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FORMULAS FOR DETERMINING THEORETICAL EFFECTS OF CERTAIN GENETIC FACTORS UPON INHERITANCE OF QUANTITATIVE CHARACTERS, WITH SPECIAL REFERENCE TO A STUDY OF A LYCOPERSICON HYBRID [1]

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INTRODUCTION

The universal importance of quantitative characters in plant breeding stresses the desirability of obtaining more information concerning the manner of their inheritance. It is particularly important to know the extent and nature of interactions between the genes differentiating the quantitative characters. Two general methods have been used to determine such interactions. In the first method the segregation of the genes differentiating the quantitative characters is determined by the direct effects that these genes produce. The second method involves the determination of linkages between genes differentiating the quantitative characters and "marker" genes differentiating more simply inherited characters.

Hayes and Harlan (*7*) [3] and Wexelsen (*30*) used the first or direct method. They determined the major genes involved in the quantitative characters and studied their interactions through certain recognizable genotypes in the segregating populations. Using the same method, Powers (*21*) separated the 27 genotypes resulting from the segregation of the three factor pairs differentiating habit of growth in crosses between varieties of *Triticum aestivum* L. and determined the nature of the interaction of the genes. A method of setting up the expected limits of fluctuation of any one genotype was given, making it possible to separate the different genotypes and thereby study directly their effects upon habit of growth.

The second method, involving linkage of marker genes with genes differentiating qualitative or simply inherited characters, was first used by Sax (*24*) and later by Griffee (*4*), Sirks (*26*), Immer (*9*), Powers (*22*), Smith (*27*), and Currence (*3*). Lindstrom (*11*, *13*, *14*, *15*, *16*) made extensive studies by this method and presented rather conclusive evidence of the existence of major factors differentiating size of fruit in the tomato.

As pointed out by Powers (*22*), the effects and interactions measured also include any that the marker genes produce as well as the effects of genes linked with them. Studies such as these, in which the marker genes are located on the same chromosome, present sev-

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[3] Italic numbers in parentheses refer to Literature Cited, p. 575.

eral problems having a bearing upon the experimental design and upon the analysis and the interpretation of the data. For example, in order to evaluate and interpret accurately the effect of the genes influencing quantitative characters, it is necessary to determine the effect of double crossing over and dominance upon the differences between the means of the genotypes. It also is necessary to determine to what extent the data measure the effect of finite regions of the chromosome and to what extent the data from backcross and from F_2 generations can be used to check each other. This paper presents formulas and their application to a solution of these problems, together with the analysis and the interpretation of data collected on number of locules from the segregates involving *Lycopersicon esculentum* Mill. (Johannisfeuer variety) and *L. pimpinellifolium* Mill. (Red Currant variety).

The formulas apply to situations in which the marker genes and the gene pair affecting the quantitative character are linked in the coupling phase and in which the genes tending to increase the magnitude of the quantitative character are recessive. The symbols used to designate the marker genes are *Gg* and *Oo*[os].

FORMULAS FOR DETERMINING THE PROPORTION OF EACH GENOTYPE

Formulas for determining the theoretical proportion of the population that each genotype constitutes are essential to the study. In the development of such formulas those given by Haldane (*6*) for the proportion of non-cross-overs, single cross-overs, and double cross-overs were used. They are as follows:

No cross-over	(*ABC*, *abc*) $(1-m)$ $(1-n)$.
Cross-over between loci of *A* and *B* only	(*aBC*, *Abc*) $m(1-n)$.
Cross-over between loci of *B* and *C* only	(*ABc*, *abC*) $n(1-m)$.
Cross-over between loci of *A* and *B* and of *B* and *C*	(*aBc*, *AbC*) mn.

In the foregoing formulas, the cross-over value for the section *A* and *B* is m and that for the section *B* and *C* is n. The same notations were used in the development of formulas for determining the theoretical proportion of the population that each genotype constitutes. Bridges and Morgan (*1*) and Morgan, Bridges, and Sturtevant (*20*) have shown that the coefficient of coincidence varies between chromosomes and for regions within chromosomes of *Drosophila*. They report that double cross-overs may occur within regions as small as 6 units and again that interference may be complete within distances of 20 units. If double crossing over were influenced only by linkage intensity, theoretically the coefficient of coincidence would be expected to have values ranging from 0 to 1. Weinstein (*29*) and Bridges and Morgan (*1*) have shown that values, for the coefficient of coincidence, higher than 1.0 do occur. In the present study the coefficient of coincidence was taken as 1 in developing the formulas for determining the theoretically expected proportion of the population having a particular genotype.

In table 1 are presented the formulas for determining the theoretical proportions of the different genotypes expected among the progeny resulting from crossing the F_1 generation to the parent recessive for the marker genes. The locus of the gene tending to increase the magnitude of the quantitative character is taken as being in that section of the chromosome delimited by the loci of the marker genes.

The last column of table 1 gives the formulas for obtaining the theoretically expected increase in the means of the different genotypes in terms of the quantitative character. Two new notations, v and d, appear in these formulas, v being the amount by which the homozygous condition of the gene pair tending to increase the magnitude of the quantitative character increases the means of the different genotypes, and d being the amount by which the heterozygous condition of the gene pair tending to increase the magnitude of the quantitative character increases the means of the different genotypes. These formulas apply to the data obtained from the progeny of the F_1 backcrossed to the parent recessive for the marker genes. The formulas fulfilling the same purpose for the F_2 generation are given in table 2 and in the tabulation on page 558.

TABLE 1.—*Formulas for determining the theoretical proportions of the different genotypes when the locus of the gene tending to increase the quantitative character is between the loci of the two marker genes* [1]

Genotype	Proportion of genotype		Increase in quantitative character
	Heterozygous	Recessive	
$Gg\,O\,o^+b$	$\frac{(1-m)(1-n)}{2}$	$\frac{mn}{2}$	$\frac{(1-m)(1-n)d+mnv}{(1-m)(1-n)+mn}$
$Gg\,o^+b\,o^+b$	$\frac{n(1-m)}{2}$	$\frac{m(1-n)}{2}$	$\frac{n(1-m)d+m(1-n)v}{m+n-2mn}$
$gg\,O\,o^+b$	$\frac{m(1-n)}{2}$	$\frac{n(1-m)}{2}$	$\frac{m(1-n)d+n(1-m)v}{m+n-2mn}$
$gg\,o^+b\,o^+b$	$\frac{mn}{2}$	$\frac{(1-m)(1-n)}{2}$	$\frac{mnd+(1-m)(1-n)v}{(1-m)(1-n)+mn}$

[1] The formulas apply to the progeny obtained from backcrossing the F_1 to the parent recessive for the marker genes.

TABLE 2.—*Formulas for determining the theoretical proportions of the different F_2 genotypes when based on the marker genes and given for the homozygous-dominant, heterozygous-dominant, and recessive conditions of a gene pair affecting the quantitative character under investigation, the locus of the gene tending to increase the quantitative character being between the loci of the two marker genes*

Genotype	Homozygous dominant	Heterozygous	Homozygous recessive
$GG\,OO$	$\frac{(1-m)^2(1-n)^2}{4}$	$\frac{mn(1-m)(1-n)}{2}$	$\frac{m^2n^2}{4}$
$GG\,O\,o^+b$	$\frac{n[(1-n)-m(2-m)(1-n)]}{2}$	$\frac{n[(1-m)-2n(1-m)(1-n)]}{2}$	$\frac{m^2n(1-n)}{2}$
$GG\,o^+b\,o^+b$	$\frac{n^2(1-m)^2}{4}$	$\frac{mn(1-m)(1-n)}{2}$	$\frac{m^2(1-n)^2}{4}$
$Gg\,OO$	$\frac{m[(1-m)-n(1-m)(2-n)]}{2}$	$\frac{n[(1-n)-2m(1-m)(1-n)]}{2}$	$\frac{mn^2(1-m)}{2}$
$Gg\,O\,o^+b$	$mn(1-m)(1-n)$	$\frac{1-2m(1-m)-2n(1-n)+4mn(1-m)(1-n)}{2}$	$mn(1-m)(1-n)$
$Gg\,o^+b\,o^+b$	$\frac{mn^2(1-m)}{2}$	$\frac{n[(1-n)-2m(1-m)(1-n)]}{2}$	$\frac{m[(1-m)-n(1-m)(2-n)]}{2}$
$gg\,OO$	$\frac{m^2(1-n)^2}{4}$	$\frac{mn(1-m)(1-n)}{2}$	$\frac{n^2(1-m)^2}{4}$
$gg\,O\,o^+b$	$\frac{m^2n(1-n)}{2}$	$\frac{m[(1-m)-2n(1-m)(1-n)]}{2}$	$\frac{n[(1-n)-m(2-m)(1-n)]}{2}$
$gg\,o^+b\,o^+b$	$\frac{m^2n^2}{4}$	$\frac{mn(1-m)(1-n)}{2}$	$\frac{(1-m)^2(1-n)^2}{4}$

Formulas for estimating the quantitative increase in the means of the genotypes, due to the effect of 1 gene pair in the F_2 generation

Genotype:	*Increase in quantitative character*
$GGOO$	$\frac{2mn(1-m)(1-n)d+m^2n^2v}{(1-m)^2(1-n)^2+2mn(1-m)(1-n)+m^2n^2}$
$GGO^{o}O^{o}$	$\frac{m[(1-m)-2n(1-m)(1-n)]d+m^2n(1-n)v}{n[(1-n)-m(2-m)(1-n)]+m[(1-m)-2n(1-m)(1-n)]+m^2n(1-n)}$
$GG^{o}O^{o}$	$\frac{2mn(1-m)(1-n)d+m^2(1-n)^2v}{n^2(1-m)^2+2mn(1-m)(1-n)+m^2(1-n)^2}$
$GgOO$	$\frac{n[(1-n)-2m(1-m)(1-n)]d+mn^2(1-m)v}{m[(1-m)-n(1-m)(2-n)]+n[(1-n)-2m(1-m)(1-n)]+mn^2(1-m)}$
$GgO^{o}O^{o}$	$\frac{[1-2m(1-m)-2n(1-n)+4mn(1-m)(1-n)]d+2mn(1-m)(1-n)v}{1-2m(1-m)-2n(1-n)+8mn(1-m)(1-n)}$
$Gg^{o}O^{o}$	$\frac{n[(1-n)-2m(1-m)(1-n)]d+m[(1-m)-n(1-m)(2-n)]v}{mn^2(1-m)+n[(1-n)-2m(1-m)(1-n)]+m[(1-m)-n(1-m)(2-n)}$
$ggOO$	$\frac{2mn(1-m)(1-n)d+n^2(1-m)^2v}{m^2(1-n)^2+2mn(1-m)(1-n)+n^2(1-m)^2}$
$ggO^{o}O^{o}$	$\frac{m[(1-m)-2n(1-m)(1-n)]d+n[(1-n)-m(2-m)(1-n)]v}{m^2n(1-n)+m[(1-m)-2n(1-m)(1-n)]+n[(1-n)-m(2-m)(1-n)]}$
$gg^{o}O^{o}$	$\frac{2mn(1-m)(1-n)d+(1-m)^2(1-n)^2v}{m^2n^2+2mn(1-m)(1-n)+(1-m)^2(1-n)^2}$

The remaining general condition is that in which the loci of the gene pair affecting the quantitative character studied are outside the *Gg–Oo*^{ob} section of the chromosome. The formulas for determining the theoretical proportions of the different genotypes in which the gene differentiating the quantitative character is located to the left of the *Gg* region are given in table 3, together with the formulas for determining the theoretically expected increase in the means of the different genotypes of the backcross generations. Corresponding formulas for the F_2 generation are given in table 4 and in the tabulation on page 560. The situation in which the gene pair is located to the right of the *Oo*^{ob} region of the chromosome is analogous to that in which the gene pair is located to the left of *Gg*, and therefore it will not be considered in detail.

TABLE 3.—*Formulas for determining the theoretical proportions of the different genotypes when the locus of the gene tending to increase the quantitative character is to the left of the loci of the marker genes* [1]

Genotype	Proportion of genotype		Increase in quantitative character
	Heterozygous	Recessive	
$GgOO^{ob}$	$\frac{(1-m)(1-n)}{2}$	$\frac{m(1-n)}{2}$	$(1-m)d+mv$
$Gg^{o}O^{ob}$	$\frac{n(1-m)}{2}$	$\frac{mn}{2}$	
$ggOO^{ob}$	$\frac{mn}{2}$	$\frac{n(1-m)}{2}$	$md+(1-m)v$
$gg^{o}O^{ob}$	$\frac{m(1-n)}{2}$	$\frac{(1-m)(1-n)}{2}$	

[1] The formulas apply to the progeny obtained from backcrossing the F_1 to the parent recessive for the marker genes.

TABLE 4.—*Formulas for determining the theoretical proportions of the different genotypes in the F_2 generation when the locus of the gene tending to increase the quantitative character is to the left of the loci of the two marker genes*

Genotype	Homozygous dominant	Heterozygous	Homozygous recessive
$GGOO$	$\frac{(1-m)^2(1-n)^2}{4}$	$\frac{m(1-m)(1-n)^2}{2}$	$\frac{m^2(1-n)^2}{4}$
$GGO_{O^{a}b}$	$\frac{n(1-m)^2(1-n)}{2}$	$mn(1-m)(1-n)$	$\frac{m^2n(1-n)}{2}$
$GG_{O^{a}b}{}_{O^{a}b}$	$\frac{n^2(1-m)^2}{4}$	$\frac{mn^2(1-m)}{2}$	$\frac{m^2n^2}{4}$
$GgOO$	$\frac{mn(1-m)(1-n)}{2}$	$\frac{n-n^2-2mn(1-m)(1-n)}{2}$	$\frac{mn(1-m)(1-n)}{2}$
$GgO_{O^{a}b}$	$\frac{m(1-m)-2mn(1-m)(1-n)}{2}$	$\frac{1-2m(1-m)-2n(1-n)+4mn(1-m)(1-n)}{2}$	$\frac{n(1-m)-2mn(1-m)(1-n)}{2}$
$Gg_{O^{a}b}{}_{O^{a}b}$	$\frac{mn(1-m)(1-n)}{2}$	$\frac{n-n^2-2mn(1-m)(1-n)}{2}$	$\frac{mn(1-m)(1-n)}{2}$
$ggOO$	$\frac{m^2n^2}{2}$	$\frac{mn^2(1-m)}{2}$	$\frac{n^2(1-m)^2}{2}$
$ggO_{O^{a}b}$	$\frac{m^2n(1-n)}{2}$	$mn(1-m)(1-n)$	$\frac{n(1-m)^2(1-n)}{2}$
$gg_{O^{a}b}{}_{O^{a}b}$	$\frac{m^2(1-n)^2}{2}$	$\frac{m(1-m)(1-n)^2}{2}$	$\frac{(1-m)^2(1-n)^2}{2}$

Formulas for estimating the quantitative increase in the means of the genotypes in the F_2 generation due to the effect of a gene pair to the left of the marker genes

Genotype:	Increase in quantitative character
$GGOO$ $GGOo^{ob}$ $GGo^{ob}o^{ob}$	$2m(1-m)d+m^2v$
$GgOO$ $GgOo^{ob}$ $Ggo^{ob}o^{ob}$	$[1-2m(1-m)]d+m(1-m)v$
$ggOO$ $ggOo^{ob}$ $ggo^{ob}o^{ob}$	$\frac{m(1-m)d+(1-m)^2v}{m+(1-m)^2}$

APPLICATION OF THE FORMULAS

DETERMINING EFFECTS DUE TO REGIONS OF THE CHROMOSOME

With the foregoing formulas available, it is possible to estimate the theoretical amounts by which the means of the different genotypes will be increased as regards the quantitative character under investigation. Also, it is desirable to know what effect varying genetic map distances between the genes affecting both the quantitative and marker characters would have upon these estimates. Therefore, the theoretical estimates of the amounts by which the means of the different genotypes would be increased were calculated for different values of m and n. Further, it is desirable to know what effect different degrees of dominance would have upon these same estimates. The effects of different degrees of dominance can be determined by considering two extreme cases; namely, that in which d equals 0 and in which d equals $v/2$. With these points considered, the theoretical estimates of the amounts by which the means of the different genotypes would be increased by a recessive gene tending to increase the quantitative character and located midway between g and o^{ob} have been calculated and are listed in table 5. The theoretical amounts, or values, are expressed as percentages of v.

First, consider the theoretical proportionate effect of this gene that will be expressed in the means of the different genotypes, when m and n each equals 0.075 and d equals 0 and when the segregates, the means of whose genotypes are being studied, have resulted from backcrossing the F_1 to the recessive parent. From table 5 it can be seen that, theoretically, the mean of the segregates having the genotype $GgOo^{ob}$ has been increased by 0.65 percent of v and that the mean of the segregates having the genotype $ggo^{ob}o^{ob}$ has been increased by 99.35 percent of v. Then the theoretical proportionate effect of the gene tending to increase the magnitude of the quantitative character that can be measured in the progeny obtained by backcrossing to the parent recessive for the marker genes is $ggo^{ob}o^{ob}-GgOo^{ob}$, or 98.7 percent. The loss of 1.3 percent may be attributed to double crossing over. This loss (table 5) increases with an increase in the values of m and n. For the same comparison in which d equals $v/2$, or the heterozygote is intermediate, the theoretical proportionate effect of the gene tending to increase the quantitative character that can be

measured by the comparison $ggo^{ob}o^{ob}-GgOo^{ob}$ is 49.34 percent of v. The difference between this value (49.34) and the same value (98.7) in which d equals 0 measures the effect of dominance, which is 49.36 percent of v. As would be expected, the greater the departure from complete dominance the smaller is the measurable proportion of the effect, in the homozygous condition, of the gene tending to increase the magnitude of the quantitative character.

TABLE 5.—*Theoretical increase in the means of the different genotypes, due to a gene located midway between g and o^{ob} and recessive to its allel located between G and O*

F_1 BACKCROSSED TO THE RECESSIVE PARENT

Genotype or phenotype	Value of d	Theoretical increase with indicated cross-over values of m and n					
		m=0.075 n= .075	m=0.150 n= .150	m=0.225 n= .221	m=0.30 n= .30	m=0.375 n= .375	m=0.450 n= .450
Genotype:		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
$GgOo^{ob}$	0	0.65	3.01	7.77	15.52	26.47	40.10
$Ggo^{ob}o^{ob}$	0	50.00	50.00	50.00	50.00	50.00	50.00
$ggOo^{ob}$	0	50.00	50.00	50.00	50.00	50.00	50.00
$ggo^{ob}o^{ob}$	0	99.35	96.99	92.23	84.48	73.53	59.90
$GgOo^{ob}$	$v/2$	50.33	51.50	53.88	57.76	63.24	70.05
$Ggo^{ob}o^{ob}$	$v/2$	75.00	75.00	75.00	75.00	75.00	75.00
$ggOo^{ob}$	$v/2$	75.00	75.00	75.00	75.00	75.00	75.00
$ggo^{ob}o^{ob}$	$v/2$	99.67	98.50	96.12	92.24	86.76	79.95
F_2 GENERATION [1]							
Phenotype:[1]							
$GgOo^{ob}$	0	0.76	3.01	6.58	11.13	16.27	21.57
$Ggo^{ob}o^{ob}$	0	47.83	45.06	41.65	37.66	33.16	28.32
$ggOo^{ob}$	0	47.83	45.06	41.65	37.66	33.16	28.32
$ggo^{ob}o^{ob}$	0	98.70	94.05	85.06	71.37	54.06	35.88
$GgOo^{ob}$	$v/2$	32.35	32.81	34.70	37.84	41.96	46.68
$Ggo^{ob}o^{ob}$	$v/2$	72.83	70.06	66.65	62.66	58.16	53.32
$ggOo^{ob}$	$v/2$	72.83	70.06	66.65	62.66	58.16	53.32
$ggo^{ob}o^{ob}$	$v/2$	99.35	96.98	92.23	84.48	73.53	59.90

[1] The homozygous dominants are included with the heterozygous dominants.

Turning to the F_2 generation in which the homozygous-dominant and heterozygous-dominant segregates have not been separated and in which d equals 0, the effects of double crossing over are not great when m and n have small values (0.075 or 0.150), and therefore a fairly accurate measure of the effect of the gene tending to increase the quantitative character is obtained by the comparison $ggo^{ob}o^{ob}-GgOo^{ob}$ (table 5). In drawing other conclusions from the F_2 data presented in table 5, it must be kept in mind that the homozygous-dominant and heterozygous-dominant segregates were not separated.

The theoretical estimates of the amount by which the means of the different genotypes will be increased in respect to the quantitative character under investigation for the F_2 generation in which the homozygous dominants and heterozygous dominants have been separated are given in table 6. More than 90 percent of the theoretical expected effect of a gene tending to increase the magnitude of the quantitative character is measured by the comparison $ggo^{ob}o^{ob}-GGOO$ when d equals 0 or $v/2$ and when the values of m and n do not exceed 0.150.

TABLE 6.—*Theoretical effects in percent upon the means of all F_2 genotypes of a gene located midway between g and o^{ob} and tending to increase the quantitative character but recessive to its allel located between G and O*

Value of *d* and genotype	Theoretical effects with indicated cross-over values of *m* and *n*					
	m=0.075 *n*= .075	*m*=0.150 *n*= .150	*m*=0.225 *n*= .225	*m*=0.30 *n*= .30	*m*=0.375 *n*= .375	*m*=0.45 *n*= .45
d=0:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
GGOO	0.01	0.09	0.60	2.41	7.01	16.08
GGOo^{ob}	.33	1.51	3.89	7.76	13.24	20.05
GGo^{ob}o^{ob}	25.00	25.00	25.00	25.00	25.00	25.00
GgOO	.33	1.51	3.89	7.76	13.24	20.05
GgOo^{ob}	1.27	5.24	11.14	17.20	21.89	24.50
Ggo^{ob}o^{ob}	49.67	48.49	46.11	42.24	36.76	29.95
ggOO	25.00	25.00	25.00	25.00	25.00	25.00
ggOo^{ob}	49.67	48.49	46.11	42.24	36.76	29.95
gg$o^{ob}o^{ob}$	98.70	94.05	85.06	71.37	54.06	35.88
d=*v*/2:						
GGOO	.65	3.02	7.77	15.52	26.47	40.10
GGOo^{ob}	25.33	26.51	28.89	32.76	38.24	45.05
GGo^{ob}o^{ob}	50.00	50.00	50.00	50.00	50.00	50.00
GgOO	25.33	26.51	28.89	32.76	38.24	45.05
GgOo^{ob}	50.00	50.00	50.00	50.00	50.00	50.00
Ggo^{ob}o^{ob}	74.67	73.49	71.11	67.24	61.76	54.95
ggOO	50.00	50.00	50.00	50.00	50.00	50.00
ggOo^{ob}	74.67	73.49	71.11	67.24	61.76	54.95
gg$o^{ob}o^{ob}$	99.35	96.98	92.23	84.48	73.53	59.90

So far the discussion has been limited to calculations in which the position of the gene tending to increase the magnitude of the quantitative character was taken as midway between the loci of the two gene pairs differentiating the qualitative character. Tables 7 and 8 give the theoretically expected amounts by which the means of the different genotypes would be increased when the values of *m* and *n* are not equal. In the examples chosen the gene pair tending to increase the magnitude of the quantitative character is taken (in genetic-map units) as being located closer to *g* than to o^{ob}. An inspection of tables 7 and 8 shows that the conclusions concerning the proportionate amounts of the effect of the gene pair (in the homozygous condition) tending to increase the magnitude of the quantitative character that is measured by the comparisons *gg$o^{ob}o^{ob}$—GgOo^{ob}* and *gg$o^{ob}o^{ob}$—GGOO* are the same as those drawn for the calculations (tables 5 and 6) made on the basis that the gene affecting the quantitative character is located midway between *g* and o^{ob}. When neither *m* nor *n* exceeded a value of 0.150 and *d* equaled 0, the comparison *gg$o^{ob}o^{ob}$—GgOo^{ob}* for the backcross and the comparison *gg$o^{ob}o^{ob}$—GGOO* for the F_2 generation measured more than 95 percent of the effect of the gene in the homozygous condition. This same statement is true for that part of the F_2 generation in which the homozygous-dominant and heterozygous-dominant segregates were separated and *d* equaled *v*/2 (table 8).

In the development of the formulas the coefficient of coincidence was taken as 1, which is seldom, if ever, the case when the values of *m* and *n* are small. Therefore, in actual experiments the effect of double crossing over upon the proportionate amount by which the means of the different genotypes would be increased probably is less than is shown by the data of tables 5 to 8, inclusive.

TABLE 7.—*Theoretical effects in percent upon the means of the different genotypes of a gene located closer to g than to o^{ob} in the section between g and o^{ob} and tending to increase a quantitative character but recessive to its allel located between G and O*

F_1 BACKCROSSED TO THE RECESSIVE PARENT

Genotype	Theoretical effects, with indicated cross-over values of m and n, where—					
	$d=0$			$d=v/2$		
	$m=0.075$ $n=.150$	$m=0.15$ $n=.30$	$m=0.30$ $n=.45$	$m=0.075$ $n=.150$	$m=0.15$ $n=.30$	$m=0.30$ $n=.45$
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
$GgOo^{ob}$	1.41	7.03	25.96	50.71	53.52	62.98
$Ggo^{ob}o^{ob}$	31.48	29.17	34.37	65.74	64.58	67.19
$ggOo^{ob}$	68.52	70.83	65.63	84.26	85.42	82.81
$ggo^{ob}o^{ob}$	98.59	92.97	74.04	99.29	96.48	87.02

F_2 GENERATION[1]

$GgOo^{ob}$	1.52	5.87	15.67	32.32	34.48	41.85
$Ggo^{ob}o^{ob}$	28.66	23.03	21.14	61.26	54.06	47.94
$ggOo^{ob}$	65.23	62.41	46.84	81.86	79.47	68.50
$ggo^{ob}o^{ob}$	97.20	86.43	54.82	98.59	92.97	74.04

[1] The homozygous-dominants are included with the heterozygous dominants.

TABLE 8.—*Theoretical effects upon the means of all F_2 genotypes of a gene located closer to g than to o^{ob} in the section between g and o^{ob} and tending to increase a quantitative character but recessive to its allel located between G and O*

Genotype	Theoretical effects, with indicated cross-over values of m and n, where—					
	$d=0$			$d=v/2$		
	$m=0.075$ $n=.150$	$m=0.15$ $n=.30$	$m=0.30$ $n=.45$	$m=0.075$ $n=.150$	$m=0.15$ $n=.30$	$m=0.30$ $n=.45$
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
$GGOO$	0.02	0.49	6.74	1.41	7.03	25.96
$GGOo^{ob}$	.44	2.05	8.93	16.45	18.10	30.17
$GGo^{ob}o^{ob}$	9.91	8.51	11.82	31.48	29.17	34.37
$GgOO$	.97	4.98	17.04	34.96	38.93	45.79
$GgOo^{ob}$	2.61	9.93	20.76	50.00	50.00	50.00
$Ggo^{ob}o^{ob}$	31.04	27.12	25.45	65.04	61.07	54.21
$ggOO$	46.95	50.17	43.07	68.52	70.83	65.63
$ggOo^{ob}$	67.55	65.85	48.59	83.55	81.90	69.83
$ggo^{ob}o^{ob}$	97.20	86.43	54.82	98.59	92.97	74.04

DETERMINING INTERACTIONS AND THEIR NATURE

In a study of the interactions of the genes affecting the quantitative characters and located at different loci of the same chromosome, it is necessary to know whether the effects of double crossing over and dominance are confounded with the interactions, and if so in what generations. As regards the interactions there are three possible situations. (1) There may not be any interaction between the genes differentiating the quantitative character. In such a case, the effects of the different gene pairs tending to increase the magnitude of the quantitative character will be strictly additive when combined. (2) Upon combination, the nature of the interaction of the genes may be such "that the effect of each factor on the genotype is dependent

upon all the other factors present, the visible effect of a certain factor being smaller the greater the number of factors acting in the same direction" (*23*). (3) The nature of the interaction of the genes differentiating the quantitative character may be such that the genes tending to increase the magnitude of the quantitative character may give greater differences over their allels in combination with genes also tending to increase the magnitude of the quantitative character than when in combination with the allels of these genes (*22*). In testing for and studying the nature of the interaction of factors the following comparison is used: $(Ggo^{ob}o^{ob}-GgOo^{ob})-(ggo^{ob}o^{ob}-ggOo^{ob})$.

Consider first the F_1 generation backcrossed to the recessive parent. In all cases, barring possible differences due to dropping of figures to the right of the decimal point, the comparison $(Ggo^{ob}o^{ob}-GgOo^{ob})-(ggo^{ob}o^{ob}-ggOo^{ob})$ equals 0. That such must be the case can be proved readily by substituting the formulas given in table 1 for the corresponding genotypes and solving the equation. Then it is apparent that neither the effects of double crossing over nor of dominance are confounded with the interactions.

Turning to the F_2 generation, it can be seen that when m and n each equals 0.075 and d equals 0 the comparison $(ggo^{ob}o^{ob}-ggOO)-(GGo^{ob}o^{ob}-GGOO)$ equals 48.71 percent and not 0 (table 6). Obviously, without the aid of table 6 the discrepancy between the differences might have been interpreted as an interaction of genes differentiating the quantitative character. The discrepancy of 48.71 percent is not directly due to the effect of double crossing over nor the degree of dominance, but is attributable to recombination of gametes containing certain single cross-overs. The discrepancies are small and consequently not so serious when the comparisons involve only the heterozygous dominants and the homozygous recessives for example the comparison $(ggo^{ob}o^{ob}-ggOo^{ob})-(Ggo^{ob}o^{ob}-GgOo^{ob})$, of table 6, in which d equals 0 and m and n each equals 0.150. The same comparisons (tables 6 and 8) in which d equals $v/2$ show that neither the effects of double crossing over nor the effects of dominance are confounded with possible effects due to interactions, but that the effects due to recombination of gametes containing certain single cross-overs are confounded with the effects due to possible interactions of genes differentiating quantitative characters. Therefore, in testing for interactions and in studying the nature of the interactions of genes differentiating quantitative characters by means of their linkage relationship with marker genes, in which all the genes involved have their loci on the same or homologous chromosomes, allowances must be made, in an interpretation of the results, for the effects due to recombination of gametes containing certain single cross-overs.

With the foregoing information available, it is possible to determine to what extent the effect of finite regions of the chromosomes is measured by following the segregation of the genes differentiating the quantitative characters by means of their linkage relations with the marker genes. To do this it is necessary to know what effect genes located to the left of the *Gg* region of the chromosome would have upon the means of the *OO*, Oo^{ob}, and $o^{ob}o^{ob}$ segregates and what effect genes located to the right of the Oo^{ob} region of the chromosome would have upon the means of the *GG*, *Gg*, and *gg* segregates. Since the two

situations are analogous, only one need be considered in detail. For example, take the case in which the additional genes affecting the quantitative character are located to the left of *Gg*. The formulas for determining the theoretical proportions of the population and the theoretically expected amounts by which the means of the different genotypes would be increased are given in tables 3 and 4 and in the tabulation on page 560. Table 3 gives the formulas applicable to the F_1 generation backcrossed to the parent recessive for the marker genes. From the column "Increase in quantitative character" it can be seen that the formulas are the same for the genotypes $GgOo^{ob}$ and $Ggo^{ob}o^{ob}$ and for the genotypes $ggOo^{ob}$ and $ggo^{ob}o^{ob}$. In the tabulation (p. 560) giving the formulas applicable to the F_2 generation, the formulas are shown to be the same for the genotypes *GGOO*, $GGOo^{ob}$, and $GGo^{ob}o^{ob}$; for the genotypes *GgOO*, $GgOo^{ob}$, and $Ggo^{ob}o^{ob}$; and for the genotypes *ggOO*, $ggOo^{ob}$, and $ggo^{ob}o^{ob}$. Such being the case, it is evident that genes located to the left of the *Gg* region of the chromosome have no effect upon the means of *OO*, Oo^{ob}, or $o^{ob}o^{ob}$ segregates when the comparisons are made within a particular genotype resulting from a segregation of the *Gg* allels. Likewise, for similar comparisons the means of the *GG*, *Gg*, and *gg* genotypes are not affected by genes having their loci to the right of Oo^{ob}.

The foregoing discussion has already largely brought out to what extent the data from the F_1 backcrossed to the parent recessive for the marker genes and the data from the F_2 generation can be used as checks against each other. The percentages tabulated in tables 5, 6, 7, and 8 show that within limits the data from one can be used in checking the conclusions drawn from the data of the other, if the formulas listed in tables 1 to 4 and in the tabulations on pages 558 and 560 are applied and the results used as an aid in an interpretation of the data.

A THREE-POINT EXPERIMENTAL DESIGN

The information derived from the foregoing tables aids materially in designing an experiment to study the nature of the interaction of genes located in the same chromosome and differentiating quantitative characters. The study of the nature of the interaction of the quantitative genes can be made by means of their linkage with marker genes. For example, consider three gene pairs serving as markers and designated as *Gg*, Oo^{ob}, and *Hh*. In addition, suppose the linkage relationship of these three gene pairs to be as follows:

$$\frac{G\ O\ H}{g\ o^{ob}\ h}$$

Further, let it be stipulated that double cross-overs do not occur within the section delimited by any two adjacent nonallelic genes. The different regions of the chromosome shall be designated as *Gg*, Oo^{ob}, and *Hh*. The design is that of a three-point experiment in which the linkage intensity is sufficient to prevent double crossing over within sections of the chromosomes delimited by adjacent nonallelic genes.

The efficacy of the three-point experiment, as a design for determining the effect of different regions of the chromosome upon the quantitative character under investigation and for determining the

nature of the interaction of the genes affecting this character by means of their linkage with marker genes, needs to be determined. Also, the relationship between the effect of the different regions (*Gg*, Oo^{ob}, and *Hh*) is important. For example, consider the population derived from the backcross of the F_1 generation to the parent recessive for all three of the gene pairs differentiating the simply inherited characters. Eight different genotypes should appear among the progeny of such a backcross, namely:

$GgOo^{ob}Hh$	$Ggo^{ob}o^{ob}hh$
$GgOo^{ob}hh$	$ggOo^{ob}hh$
$Ggo^{ob}o^{ob}Hh$	$ggo^{ob}o^{ob}Hh$
$ggOo^{ob}Hh$	$ggo^{ob}o^{ob}hh$

The problem involved is how the comparison between the different genotypes should be interpreted. For example, the difference $GgOo^{ob}Hh - Ggo^{ob}o^{ob}Hh$ could be due to a gene affecting the quantitative character under investigation and located in either the section delimited by *Gg* and Oo^{ob} or the section delimited by Oo^{ob} and *Hh*. Also, the effect could be due to the loci designated as *O* and o^{ob}. If the gene or genes having an effect upon the quantitative character are located between *Gg* and Oo^{ob}, the *Gg* region of the chromosome, as has been previously shown, would also have an effect upon the quantitative character due to the same gene or genes. For example, suppose that the difference between the gene having an effect upon the quantitative character in the heterozygous condition had a value of 0, as compared with its allel in the homozygous recessive condition having a value of 4. Under such specifications, the difference between $Ggo^{ob}o^{ob}Hh$ and $GgOo^{ob}Hh$ would be 2 and the difference between $ggOo^{ob}Hh$ and $GgOo^{ob}Hh$ would be 2 also. Their sum would give the total effect of the gene considered in the homozygous recessive condition. As has been shown in a previous section of this paper, the gene pair whose effect is being studied need not be located midway between the gene pairs *Gg* and Oo^{ob} for the differences to add up to the total effect of the gene in the homozygous condition. If the gene occupied any locus in the $Gg-Oo^{ob}$ section of the chromosome, the results would be the same, under the stipulations, namely, that double cross-overs do not occur in the section between *Gg* and Oo^{ob}. It should not be concluded that the comparison $(Ggo^{ob}o^{ob}Hh - GgOo^{ob}Hh) + (ggOo^{ob}Hh - GgOo^{ob}Hh)$ measures only the effects of genes located between the gene pairs *Gg* and Oo^{ob}, as any genes to the left of *Gg* and to the right of Oo^{ob} and to the left of *Hh* would have an effect. However, it is apparent that the comparison $(Ggo^{ob}o^{ob}Hh - GgOo^{ob}Hh) + (ggOo^{ob}Hh - GgOo^{ob}Hh)$ must include the total effect of the Oo^{ob} region, and it is equally apparent that the effect of two regions, whose effects have been obtained in such a manner, can be summed to give the total effect of the two regions, provided an interaction of the genes differentiating the quantitative characters does not exist.

This leads to the problem of testing for and evaluating the interactions. It can be seen from the foregoing examples that the comparison $(Ggo^{ob}o^{ob}Hh - GgOo^{ob}Hh) - (ggo^{ob}o^{ob}Hh - ggOo^{ob}Hh)$ does not necessarily represent a test for an interaction, because, if only one gene pair differentiating the quantitative character being considered were involved, the value obtained would be 0 (see table 5, F_1 backcrossed to the recessive parent), which is the same value as would be

obtained if more than one gene pair were affecting the quantitative character and no interaction of the genes existed. However, the comparison $(ggOo^{ob}Hh - GgOo^{ob}Hh) - (ggOo^{ob}hh - GgOo^{ob}hh)$ does constitute a test for interactions and does offer a means of testing the nature of these interactions, provided the *Hh* region of the chromosome has been shown to have an effect, because the differences between *gg* and *Gg* in such a comparison are not affected by genes located to the right of Oo^{ob} other than through possible interactions. Therefore, it is obvious that the three-point experiment as outlined is adapted to studying the nature of the interaction of genes differentiating quantitative characters.

In conclusion it should be pointed out that, even though more than three gene pairs differentiating more than three marker characters are involved in the study, the three-point experiment is very useful, since larger numbers of individuals will be available for estimating the value of each genotype than if an attempt were made to study all regions simultaneously. However, more information concerning the interactions can be obtained, if adequate numbers are available to estimate the means for each genotype, by considering all regions of the chromosomes in one extensive analysis. But such a utopian situation as adequate numbers for all genotypes possible from a segregation of more than three linked gene pairs is seldom realized in working with plant material.

NUMBER OF LOCULES IN TOMATOES

The purpose of the preceding formulas and tables is to aid in the interpretation of data on the inheritance of quantitative characters. They apply to certain cases in which regions of homologous chromosomes are being studied. A study on the inheritance of number of locules in tomatoes offered an opportunity for the practical application of the information derived from the foregoing study. The results obtained are reported herewith.

The study on the inheritance of number of locules in tomatoes was made from a cross involving *Lycopersicon esculentum* var. Johannisfeuer (the many-loculed parent) and *L. pimpinellifolium* var. Red Currant (the few-loculed parent). Data were taken from the F_2 generation, the F_1 generation backcrossed to both parents, the F_1 generation, and both parents. Owing to the limited number of F_1 plants available, they were grown in a separate randomized experiment with both parents. In the F_2 generation, both backcrosses and the parents were grown in a randomized block experiment, consisting of rows having 24 plants each, with 3½ feet between plants within the row and the same distance between rows. The rows per block were distributed as follows: The F_2 generation, 16 rows; the backcross to Red Currant, 4 rows; the backcross to Johannisfeuer, 12 rows; Johannisfeuer, 2 rows; and Red Currant, 3 rows. The rows were randomized within blocks by the use of Tippett's (*28*) tables, and a total of 9 blocks was used in the analysis. The method of analysis was that given by Powers (*22*), in which the *t* test was used to determine statistically significant differences.

Of importance in an interpretation of the data are (1) dominance of the genes affecting the quantitative character as well as those used as marker genes, (2) intensity of the linkage relationship of the genes

used as markers, and (3) number of factor pairs involved in differentiating the characters used as markers.

As previously stated, the quantitative character under consideration was number of locules. Depth of grooves and shape of fruit were the two characters whose segregation in the two different generations made it possible to determine the major gene pairs involved in their differentiation and the linkage relationships. The characters number of locules and depth of grooves are shown in figure 1. It will be remembered that the F_1 generation plants were grown in comparison with plants of both parents and are not directly comparable with the parents grown for comparison with the F_2 and backcross generation plants. The data given in explanation of figure 1 are comparable. The Johannisfeuer parent averaged 9.60 locules per fruit, the F_1 generation 2.40 locules per fruit, and the Red Currant parent 2.00 locules

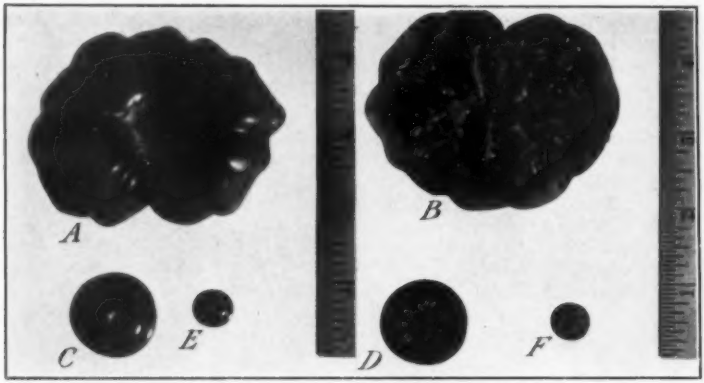

FIGURE 1.—Tomato hybrid and parents: *A*, *B*, Johannisfeuer parent; *C*, *D*, F_1 generation; *E*, *F*, Red Currant parent. Mean number of locules: Johannisfeuer, 9.60; F_1 generation, 2.40; Red Currant, 2.00. Left (*A*, *C*, *E*) shows smooth type dominant.

per fruit. The differences between the mean number of locules for Johannisfeuer and the mean number of locules for either the F_1 generation or the Red Currant parent are statistically significant; but the difference between the mean number of locules for the F_1 generation and the mean number of locules for the Red Currant parent does not reach statistical significance if odds of 19 : 1 against the deviations noted as due to the probable errors of random sampling are accepted as a criterion. It is apparent that the character few locules per fruit is completely dominant or nearly so. The photographs of the three fruits to the left in figure 1 (*A*, *C*, *E*) show that smooth type of fruit is almost completely dominant to fruit with deep grooves.

Next, consider shape of fruit (fig. 2). To facilitate the comparison of shapes, the photographs of the three fruits have been brought to approximately the same diameter. It can be seen that the fruit of the Red Currant parent is essentially round, that of the F_1 generation somewhat oblate, and that of the Johannisfeuer parent decidedly oblate. It was possible to separate the homozygous dominants from

the heterozygous dominants in the generations segregating for fruit shape; but the separation is not shown in the F_2 generation because of the inadequate number of some genotypes.

The segregation for depth of grooves and shape of fruit will be considered next. The ratio of smooth-fruited plants to those having deep grooves was 2,508:871 in the F_2 generation and 1,272:1,229 among the progeny of the F_1 backcrossed to Johannisfeuer; all of the plants from the F_1 generation backcrossed to Red Currant had smooth fruits. As to shape of fruit, the ratio of round fruit to somewhat oblate fruit to decidedly oblate fruit was 851:1,651:877 for the F_2

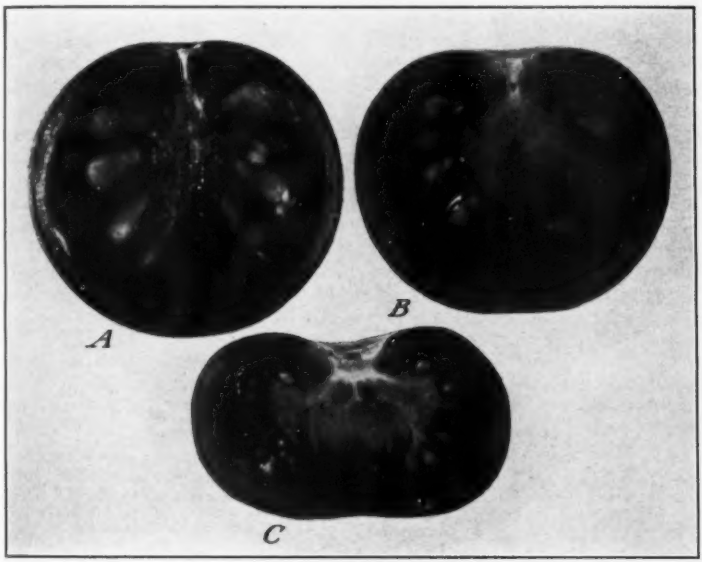

FIGURE 2.—Tomato hybrid and parents. *A*, Red Currant parent; shape, round. *B*, F_1 generation; shape, somewhat oblate. *C*, Johannisfeuer parent; shape, decidedly oblate.

generation; the ratio of somewhat oblate to decidedly oblate was 1,249:1,252 for the progeny of the F_1 backcrossed to Johannisfeuer; and the ratio of round to somewhat oblate was 410:438 for the F_1 backcrossed to Red Currant. It is apparent that depth of grooves and shape of fruit are each differentiated by one major factor pair. Lindstrom (*12*, *13*) believes that a series of multiple allels governs fruit shape in tomatoes. Since he found that round and oval shape of fruit segregated as expected on the basis of a one-factor pair, and since the segregation for round and oblate shape of fruit reported herein is that expected on the basis of a one-factor pair, the present study offers further support for the deductions made by Lindstrom.

Turning to the linkage intensities, the major factor pair differentiating depth of grooves was found to be linked with the major factor pair differentiating shape of fruit, with 18 ± 0.76 percent of crossing over in the F_2 generation and 15 ± 0.71 percent of crossing over among

the progeny obtained from crossing the F_1 generation back to Johannisfeuer. Immer's (*10*) formulas were used in calculating the crossover values and standard errors. The linkage was in the coupling phase. The symbols used to designate the genes involved were as follows:

G—symbol used for the gene differentiating smooth from deeply grooved type of fruit.
g—symbol used for the allel of *G*.
O—symbol used for the gene differentiating round from oblate type of fruit.
o^{ob}—symbol used for the allel of *O*.

With the foregoing data on the inheritance of the different characters, it is possible to determine the effect of the *Gg* and Oo^{ob} regions of the chromosome upon number of locules and to ascertain whether the genes located in the two regions interact and, if so, the nature of the interaction. The linkage intensities of 18 percent for the F_2 generation and 15 percent for the backcross generation, the almost complete dominance exhibited by the F_1 generation as compared to the parents, and the almost complete lack of difference between the means of the $Ggo^{ob}o^{ob}$ and $ggOo^{ob}$ genotypes make it appear that those data of table 5, column 3, in which *d* equals 0 furnish a satisfactory estimate of the effect that the maximum expected amount of double crossing over and the degree of dominance would have upon the means of the different genotypes, and therefore they should be of value as a guide in interpreting the data given in tables 9 and 10. To facilitate comparisons, the values referred to (table 5) have been copied in column 3 of table 9.

TABLE 9.—*Mean number of locules for the different generations, genotypes, and parents of the cross Lycopersicon esculentum var. Johannisfeuer × L. pimpinellifolium var. Red Currant*

Generation, genotype, and parent	Obtained mean number	Theoretical percentage of t [1]	Generation, genotype, and parent	Obtained mean number	Theoretical percentage of t [1]
F_2 generation: [2]			F_1 backcrossed to Red Currant:		
$GgOo^{ob}$	2.52	0.76	Oo^{ob}	2.21	
$Ggo^{ob}o^{ob}$	3.51	47.83	$o^{ob}o^{ob}$	2.36	
$ggOo^{ob}$	3.52	47.83	Parents:		
$ggo^{ob}o^{ob}$	5.22	98.70	Red Currant	2.04	
F_1 backcrossed to Johannisfeuer:			Johannisfeuer	10.27	
$GgOo^{ob}$	3.61	.65			
$Ggo^{ob}o^{ob}$	5.04	50.00			
$ggOo^{ob}$	5.03	50.00			
$ggo^{ob}o^{ob}$	7.48	99.35			

[1] See table 5, column 3, in which $d=0$.
[2] The homozygous dominants and heterozygous dominants are not separated.

Column 3 of table 9 shows that, on the basis of only one gene pair differentiating plants with few-loculed fruits from plants with many-loculed fruits, the means of the $Ggo^{ob}o^{ob}$ and $ggOo^{ob}$ genotypes should be identical. From column 2 of table 9 it can be seen that the means for these two genotypes in both the F_2 and backcross generations are almost identical. It should be pointed out that the differences between means of these two genotypes in both the F_2 and backcross generations were smaller than would be expected on the basis of the *t* test. As the variance of error was estimated for within genotypes

and blocks, ignoring replication, the discrepancy may be due to the fact that differences between means of replications were not removed from an estimate of the variance. The reason for not removing the

TABLE 10.—*Main effects and interactions of different regions of the chromosome and of different generations upon number of locules in the cross Johannisfeuer × Red Currant* [1]

Item	Differences for within generations and genotypes			
	F_2		B_1[2]	
Main effects:				
Regions:				
$gg-Gg$	Oo^{ob} 1.00	$o^{ob}o^{ob}$ 1.71	Oo^{ob} 1.42	$o^{ob}o^{ob}$ 2.44
$o^{ob}o^{ob}-Oo^{ob}$	Gg .99	gg 1.70	Gg 1.43	gg 2.45
	Genotype			
Generations:				
$^2B_1-F_2$	$GgOo^{ob}$ 1.09	$Ggo^{ob}o^{ob}$ 1.53	$ggOo^{ob}$ 1.51	$ggo^{ob}o^{ob}$ 2.26
	First order			
Interactions:				
$Gg\times Oo^{ob}$	F_2 .71	B_1[2] 1.02		
$Gg\times$generations	Oo^{ob} .42	$o^{ob}o^{ob}$ .73		
$Oo^{ob}\times$generations	Gg [3] .44	gg [3] .75		
	Second order			
$Gg\times Oo^{ob}\times$generations	[4] .31			

[1] The t test gives a value of $P<0.01$ for all values not otherwise designated.
[2] B_1 designates the progeny from the F_1 backcrossed to the recessive parent, Johannisfeuer.
[3] $P>0.01$ but <0.02.
[4] $P>0.05$.

variance due to differences between means of replications was that the number of plants of some genotypes was small for the replications. The fact that the means of the $Ggo^{ob}o^{ob}$ and $ggOo^{ob}$ genotypes are almost identical would indicate that only one gene pair located midway between Gg and Oo^{ob} affecting number of locules is segregating in this cross. Such being the case, certain conclusions would necessarily follow. The difference $gg-Gg$ should equal the difference $o^{ob}o^{ob}-Oo^{ob}$; the differences should be approximately the same for all genotypes and generations, or, in other words, there would be no interactions and the values listed under interactions in table 10 should not be statistically significant; there should not be any statistically significant differences between the means of the F_2 and backcross generations as regards the $ggo^{ob}o^{ob}$ genotype; and finally the mean of the $GgOo^{ob}$ genotype should be a very close approximation of the mean of the Red Currant parent and the mean of the $ggo^{ob}o^{ob}$ genotype should approximate very closely that of the Johannisfeuer parent.

To facilitate a study of whether the obtained data meet the foregoing specifications, the differences were tabulated in table 10. The

differences between allels and the differences between generations are statistically significant. The latter fact is contrary to the specification that there should be no differences between generations as regards the $ggo^{ob}o^{ob}$ genotype. Of the differences listed under interactions, all of the first-order interactions are statistically significant, whereas the triple interaction is not. This is contrary to the specification that none of the interactions of table 10 should be statistically significant. Finally, refined statistical analysis is not necessary to show that the two means for the $GgOo^{ob}$ genotype are significantly larger than the mean of the Red Currant parent and that the two means for the $ggo^{ob}o^{ob}$ genotype are smaller than the mean of the Johannisfeuer parent. Clearly, the segregation of a single factor pair will not explain the data. Some other interpretation must be sought.

From table 10, it can be seen that the differences between the means given for the generation obtained by backcrossing the F_1 to the Johannisfeuer parent and the means given for the F_2 generation are nearly equal to the differences for the comparisons $gg-Gg$ and $o^{ob}o^{ob}-Oo^{ob}$. This shows that at least one other gene pair, not closely associated with the *Gg* or Oo^{ob} regions of the chromosome, has an effect upon number of locules and is segregating in this cross. Likewise, the fact that the interaction $Gg \times Oo^{ob}$ is statistically significant shows that more than one locus of the chromosome carrying the allels *Gg* and Oo^{ob} is affecting number of locules. From these results it is very evident that at least three gene pairs affecting number of locules are segregating in this cross.

Such being the case further consideration should be given to the fact that the differences ($gg-Gg$) and ($o^{ob}o^{ob}-Oo^{ob}$) were so nearly equal, indicating that only one major factor pair affecting number of locules was segregating in the cross studied. Yeager (*31*) has shown that in the material with which he worked a major factor pair affecting number of locules was linked with the gene pair differentiating shape of fruit. However, his many-loculed parents had from 4 to 5 locules, whereas in the present study the many-loculed parent averaged 10.27 locules per fruit. The few-loculed parents had from 2 to 3 locules, as did the few-loculed parents in the present study. From these considerations, it is apparent that Johannisfeuer, the many-loculed parent involved in the cross reported herein, probably is not of the same genetic constitution as the many-loculed parents studied by Yeager. However, since Yeager has shown that a major factor pair affecting number of locules is linked with a gene pair differentiating shape of fruit, and since the writer found a major effect linked with shape of fruit, it seems probable that Johannisfeuer carries the same gene pair affecting number of locules that was reported for No. 15 and Bison by Yeager (*31*). Furthermore, the fact that the differences ($gg-Gg$) and ($o^{ob}o^{ob}-Oo^{ob}$) are so nearly equal is an indication that this gene pair, *Lclc* (Yeager, *31*) is located midway between the *Gg* loci and the Oo^{ob} loci of the chromosome. Then, the data (as regards chromosome 1 of the tomato) obtained in the cross Johannisfeuer $\times$ Red Currant can be explained by the *Lclc* gene pair plus other gene pairs located in the $Gg-Oo^{ob}$ section of the chromosome or located to the right or left of this section or both, and balanced numerically or qualitatively or both, so as to give the results noted.

Since it has been shown that at least three gene pairs affecting number of locules are segregating in the cross Johannisfeuer × Red Currant, the nature of the interactions of the genes associated with the *Gg* and *Oo*^{ob} regions of the chromosome can be determined. However, before making an interpretation of the data it is desirable to have some findings of previous investigators in mind. Mangelsdorf and Fraps (*19*) found that the vitamin A units per gram increase approximately 2.25 for each additional *Y* gene. Groth (*5*) as early as 1915 believed that a geometric progression was important in the inheritance of quantitative characters. Lindstrom (*17*), from studies to determine the nature of the interaction of the genes affecting size of fruit involving crosses between species of *Lycopersicum*, realized clearly the importance of not confounding the interaction between pairs of allels with dominance and the probability that the nature of the interactions of the genes may be such as to result in a geometric progression. His conclusions (*17, p. 10*) follow:

> In concluding, the writer does not mean to imply that geometric action of genes for quantitative characters is not an important aspect. On the contrary, such action is to be expected. But he does wish to point out that genes for quantitative characters are undoubtedly similar in behavior to qualitative genes. Since the latter exhibit dominance almost universally the same is true of the former; and the mere fact that quantitative character data seem to fit a logarithmic curve does not necessarily rule out dominance.

Houghtaling (*8*), working with cell number and cell expansion in the tomato fruit, came to the conclusion that a geometric progression should be involved in the inheritance of size of fruit. Sinnott (*25*), MacArthur and Butler (*18*), and Charles and Smith (*2*), from a study of the means and frequency distribution of the parents and F_1 and F_2 generations, interpreted the results from size inheritance studies on the basis of a geometric progression. Rasmusson's (*23*) interaction hypothesis is based on the assumption that the effect of a certain factor may be smaller the greater the number of factors acting in the same direction. Powers (*21*), working with habit of growth in *Triticum*, and Rasmusson (*23*), working with earliness of maturity in *Pisum*, obtained data that would support the interaction hypothesis. Powers (*22*) found that in general the nature of the interaction of the factors affecting weight of seed per plant, number of spikes per plant, height of plant, and length of awn in segregates from hybrids involving varieties of *Hordeum vulgare* was such that the effect of certain genes was larger the greater the number acting in the same direction. From this review of literature it may be concluded that at least four types of interactions of the genes, as measured by end products, exist. One would be such that the effects, as measured by end results, would be less than arithmetically cumulative; another would be such that the effects would be arithmetically cumulative; another would be such that the effects would be somewhat more than arithmetically cumulative; and the last would be such that the effects would be geometrically cumulative.

The fact that the values for the first-order interactions (table 10) are statistically significant shows that the genes affecting number of locules do interact, and the differences listed under allels and generations in table 10 show the nature of the interaction, as, for example, the interaction *Gg* × *Oo*^{ob}, of which the values 0.71 and 1.02 are due to the increased differences between the allels within the homozygous

recessive genotypes. Likewise, the interactions *Gg* × generations and *Oo*^{ob} × generations, with the values 0.42 and 0.73 and the values 0.44 and 0.75, respectively, are due to the increased differences between the generations within the homozygous recessive genotypes. It will be remembered that the genes favorable to an increase in number of locules are linked with the recessive marker genes. Then, it can be concluded that the nature of the interaction is such that the genes favorable to an increased number of locules gave greater differences over their allels in combination with genes for a higher number of locules than they did in combination with genes for a lower number of locules. In fact, the nature of the interactions of the genes was such as to be geometrically cumulative.

It is not possible to predict the exact number of gene pairs involved in differentiating number of locules; but the rather consistent increase in locule number from that of the Red Currant parent to that of the Johannisfeuer parent and the data, already presented, showing that at least three gene pairs affecting locule number must be segregating during sporogenesis of the F_1 generation of the cross Johannisfeuer × Red Currant make it appear that the parents differ by a large number of genes.

SUMMARY AND CONCLUSIONS

Formulas were developed for determining the theoretical effect of double crossing over and dominance upon the differences between the means of the genotypes involved in inheritance of quantitative characters. These formulas apply only under the conditions stipulated in the text.

A gene having an effect upon a quantitative character and located between the loci of the two marker genes will have an influence upon both regions of the chromosome, and, if the linkage between the two marker genes is sufficient to prevent double crossing over, the summation of the effect for the two regions will include the total effect of the gene pair influencing the quantitative character. However, in making the comparison both regions should be taken into consideration simultaneously; for example, *ggo*^{ob}*o*^{ob}—*GgOo*^{ob}.

By means of the three-point method of analysis in those cases in which double crossing over does not occur in the section of the chromosome delimited by adjacent marker genes, it is possible to determine the individual effect of regions of the chromosome upon the quantitative character under investigation. However, the nature of possible interactions between genes closely linked and located within any one region cannot be readily determined.

A region of a chromosome is designated by a pair of marker genes such as *Gg*, and a section of a chromosome by two adjacent pairs of marker genes such as *Gg–Oo*^{ob}. As previously pointed out by Powers (*22*), the regions are not fixed but vary in length according to the positions of the chiasmata; but, as has been shown, their effects can be determined and logically summed by using the three-point method of analysis. The same is true for the two-point method of analysis, if only two different regions of the chromosome are being studied.

In the data obtained from the progeny resulting from backcrossing the F_1 generation to the parent recessive for the marker genes, neither

the effects due to double crossing over nor the effects due to dominance are confounded with possible interactions. Likewise, effects of double crossing over and dominance are not confounded with possible interactions in data collected from an F_2 generation, but the effects due to recombination of gametes containing certain single cross-overs are confounded with the interactions. Therefore, tables such as 6 and 8, giving the theoretical values, aid materially in an interpretation of the data.

Double crossing over decreases the proportion of the effect due to the gene tending to increase the quantitative character that is measured. Likewise, the measurable proportionate effect decreases with a decrease in the degree of dominance.

In a study involving number of locules for the segregates of the cross *Lycopersicon esculentum* var. Johannisfeuer × *L. pimpinellifolium* var. Red Currant, the combined effect of the genes tending to increase the number of locules per fruit for the *Gg* and Oo^{ob} regions of the chromosome was 3.87 locules per fruit for the progeny obtained by backcrossing the F_1 generation to the parent recessive for the marker genes and the difference $ggo^{ob}o^{ob} - GgOo^{ob}$ was 2.70 locules per fruit for the F_2 generation.

The interactions *Gg* × Oo^{ob}, *Gg* × generations, and Oo^{ob} × generations were statistically significant. The nature of the interactions was such that the effect of the genes tending to increase number of locules per fruit was geometrically cumulative for "between means" of genotypes and for "between means" of generations for the different genotypes. These findings are in accordance with those reported by Powers (*22*) for the nature of the interaction of factors affecting weight of seed per plant, number of spikes per plant, height of plant, and length of awn in segregates from hybrids involving varieties and species of *Hordeum*.

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EFFECT OF MOISTURE, FERTILITY, AND FERTILIZER PLACEMENT ON ROOT ROT OF CANNING PEAS IN WISCONSIN[1]

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INTRODUCTION

The importance of high soil fertility in the reduction of damage to peas caused by *Aphanomyces euteiches* Drechsler was first pointed out at the New Jersey Agricultural Experiment Station in 1928 (*4, Rept. 49*).[3] In subsequent reports (*2*; *4, Repts. 51, 56, 57; 5; 6; 7*) these studies, which were carried out by Haenseler, repeatedly confirmed the earlier findings and showed that the mineral nitrogenous components of the fertilizer mixtures used were the most influential ones. The amounts of fertilizer applied were usually from 1,000 to 2,000 pounds per acre. Recently Geach in Australia showed in pot experiments in the greenhouse a measurable reduction in the amount of root rot in peas when nitrogenous fertilizers were added to infested soil.

The present paper reports the results of similar studies conducted in Wisconsin, a preliminary report of part of which has already been made (*8*). Root rot is most destructive in this State on Colby silt loam, a soil of high water-holding capacity. The disease is, of course, most serious in seasons when rainfall is plentiful during May, June, and July in accordance with the influence of soil moisture on the disease demonstrated by Jones and Drechsler (*3*). Since the usual fertilizer applications in this section are much smaller (usually 200 to 500 pounds per acre) than in New Jersey it became of interest to know whether such applications have any appreciable effect on the disease, and whether any relief from the latter may be expected without resorting to larger and otherwise unnecessary rates.

EXPERIMENTAL RESULTS

GREENHOUSE EXPERIMENTS

Colby silt-loam soil from a root-rot-infested field near Owen, Wis., was placed in a greenhouse bench to a depth of about 5 inches. Half of the area was left unfertilized while the remainder was supplied with 4–16–4 fertilizer at the rate of 500 pounds per acre, spread broadcast and worked into the upper 3 inches of the soil. Seed of three varieties—Bruce, Alaska, and Surprise—were planted in rows. The fertilized and unfertilized areas were further divided each into two equal portions. In one of each the soil was moistened only enough to permit germination and fair growth; in the other the soil was kept at a relatively high moisture level by frequent watering. Part of the plants were removed at 32 days and the remainder at 39 days.

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[2] Died September 14, 1939.
[3] Italic numbers in parentheses refer to Literature Cited, p. 590.

After the roots and lower stems were examined the plants were sorted into three groups—healthy or slight, moderate and severe—according to the degree of root rot.

When the results from the dry and the moist soils are compared (table 1) it is clear that the disease was almost completely suppressed in the former. This is in accord with the common observation on Colby silt loam in central Wisconsin where root rot is difficult to find in dry seasons and is usually very destructive in moist seasons. The reduction of the disease in fertilized moist soil as compared with that in unfertilized moist soil was very striking, showing the same trend of result as that reported on New Jersey soils.

TABLE 1.—*The effect of soil moisture and fertility on the occurrence of root rot in three varieties of peas grown in the greenhouse*

Fertilizer treatment	Variety of pea	Interval after planting	Plants in various disease groups in—					
			Dry soil			Moist soil		
			Healthy to slight	Moderate	Severe	Healthy to slight	Moderate	Severe
		Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
None	Bruce	32	100	0	0	29	0	71
		39	100	0	0	2	5	93
	Alaska	32	98	2	0	44	29	27
		39	100	0	0	0	0	100
	Surprise	32	95	0	5	37	8	55
		39	100	0	0	3	1	96
Average			98.8	.3	.8	19.2	7.2	73.7
4-16-4 500 pounds per acre.	Bruce	32	97	0	3	97	0	3
		39	100	0	0	93	0	7
	Alaska	32	100	0	0	79	21	0
		39	100	0	0	97	0	3
	Surprise	32	100	0	0	87	13	0
		39	100	0	0	89	1	10
Average			99.5	0	.5	90.3	5.8	3.8

Two successive plantings were made on the same soil without further fertilization. For these tests all the soil was maintained at a high moisture level. Five other canning varieties were included in one or the other of these trials. The results (table 2) have the same trend for each of the varieties. In all cases more than 60 percent of the plants were severely diseased in the unfertilized soil, with an average of 79 percent; in only 2 out of 10 instances were more than 30 percent of the plants severely diseased in the fertilized soil, while the average was 19 percent. Thus one fertilizer application, under greenhouse conditions, was sufficient to reduce markedly the damage from root rot in three successive crops of peas, each grown to about the first-blossom stage.

It is well at this point to describe the appearance of fertilized and unfertilized plants at the end of the experiments. Representative individuals of the variety Bruce from each treatment in moist soil in the first experiment are shown in figures 1 and 2.

The striking difference in the size of plants shown in figure 1 is obviously more than fertilizer effect. The comparison of root systems

FIGURE 1.—Peas of the variety Bruce grown in the greenhouse on root-rot-infested Colby silt-loam soil, collected 32 days after planting: *A*, Plants grown on a portion of the soil that had been treated with 500 pounds per acre of 4–16–4 fertilizer; *B*, plants from the unfertilized soil. (See fig. 2.)

in figure 2 illustrates the difference in disease development. Fertilized plants are not in any sense free from infection. In the unfertilized plants the degree of invasion and rootlet destruction is much more

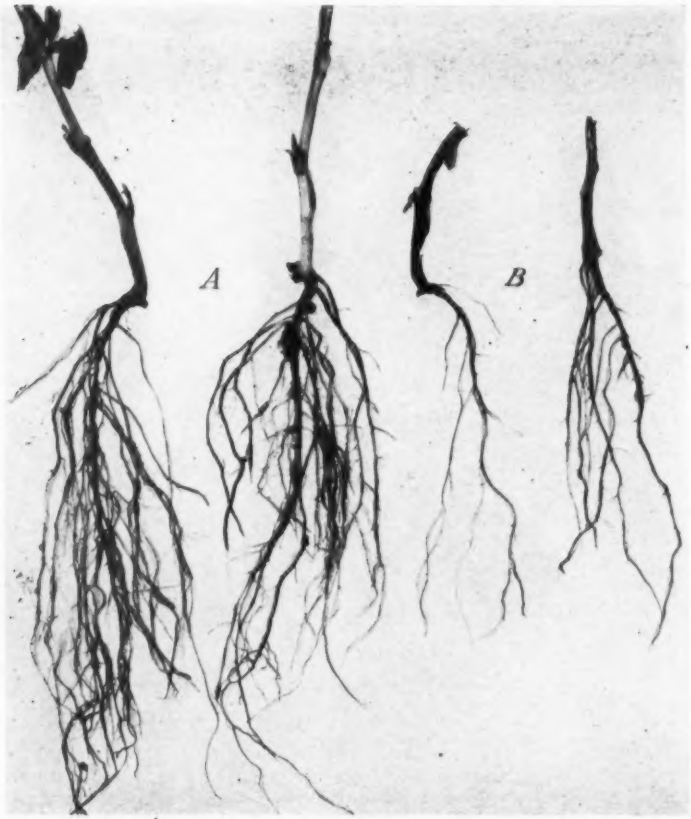

FIGURE 2.—Representative plants from the fertilized (*A*) and unfertilized (*B*) groups shown in figure 1. Note the relative severity of root rot in the two lots.

severe, while the progress of the fungus into the lower stem and the decay of the cortex in that region are also greater. It is difficult to estimate the extent of replacement of rootlets in the two differentyl treated plants. In the main the basic difference seems to lie in the fact that invasion by the fungus is much less aggressive in the roots of the fertilized plants because of the greater resistance of these plants. There seems to be no question of this fact insofar as the subterranean portions of the stems are concerned.

Fertilizer trials in the field on Colby silt loam were begun in 1932. During this and succeeding seasons up to and including 1937 little was accomplished in the way of tangible results because of the general

lack of severe root rot. This was correlated, of course, with the relatively low precipitation during the critical periods for root-rot development. In 1938, very favorable weather for root rot occurred. In heavily infested fields damage was severe. The relation of fertilizer application to severity of the disease was brought out very definitely in fertilizer trials conducted on Colby silt loam at the Branch Experiment Station at Marshfield, Wis.

TABLE 2.—*The effect of fertilizer upon the occurrence of root rot in several varieties of peas grown in the greenhouse*

Variety	Number of planting following treatment	Severely diseased plants in—		Variety	Number of planting following treatment	Severely diseased plants in—	
		Unfertilized soil	Fertilized soil			Unfertilized soil	Fertilized soil
		Percent	*Percent*			*Percent*	*Percent*
Alaska	Second	69	11	Perfection	Second	95	19
	Third	66	20	Prince of Wales	Third	63	6
Surprise	Second	73	24	Wisconsin Early Sweet	do	65	35
	Third	90	34				
Bruce	Second	97	9				
Green Admiral	do	80	5	Average		79	19
Ashford	do	87	28				

FIELD EXPERIMENTS

The Marshfield experiment was one of a series, all of which were identical in plot design, conducted by the junior author, on five soil types in various locations in Wisconsin. Root rot was noticeable only in the plot at Marshfield. The largest part of the area used for this experiment had been used for peas 3 years previously (1935). Although root rot was not serious in that year on this field, it should be borne in mind that weather conditions in 1935 were not favorable to the disease. The remainder of the 1938 experimental area had not grown peas for a much longer period. Alaska peas were planted on May 13. At this time the upper 6 inches of soil contained 35 percent of moisture, an amount much above normal when compared with previous moisture records secured at planting time at the Marshfield station. With frequent and often heavy rains following the time of planting (2.54 inches during the first 7 days after planting) root rot appeared early, and at blossom time serious damage was already evident. It was clearly seen at this date that root rot was much more severe in the part of the field that had grown peas in 1935.

When the plants were in the early-blossom stage random samples were taken from each of the plots. The average height of the plants was recorded and after the soil had been washed from the roots carefully, the percentage of severely damaged individuals was determined. In table 3 are presented the data obtained together with the order of arrangement and the various treatments. Two formulas of fertilizer, one with nitrogen (2–12–6) and one without (0–20–10) were used on duplicate plots. The first of these was used at two rates (200 and 300 pounds per acre). All fertilizer was applied at the time of planting with a combination fertilizer-grain drill, by means of which the material could be placed (1) in the drill row directly with the seed, (2) in a furrow 1½ inches to the side of and at the same level as the seed, or (3) in a shallow furrow directly over the seed, from which it was

separated by about an inch of soil. Plots 15, 16, 17, and 18 were in that part of the field on which peas had not been grown for several years, and in which root rot was least severe.

TABLE 3.—*The effect of fertilizer applications on vine height and on severity of root rot of Alaska peas grown on Colby silt loam at Marshfield, Wis., 1938*

Plot No.	Fertilizer treatment			Condition at blossom period	
	Formula	Amount per acre	Placement with relation to seed	Average vine height	Severely diseased plants
		Pounds		*Centimeters*	*Percent*
1	2-12-6	200	With	36	27.3
2	2-12-6	200	Side	35	17.9
3		0		34	76.5
4	2-12-6	300	With	39	21.4
5	2-12-6	300	Side	35	44.1
6	2-12-6	300	Over	40	30.8
7		0		28	84.4
8	0-20-10	200	Side	30	63.6
9	0-20-10	200	With	33	61.1
10	2-12-6	200	Side	30	71.8
11	2-12-6	200	With	41	20.4
12		0		30	55.6
13	0-20-10	200	Side	30	46.7
14	0-20-10	200	With	34	41.7
15[1]	2-12-6	300	do	42	8.3
16[1]	2-12-6	300	Side	39	12.5
17[1]		0		38	14.3
18[1]	2-12-6	300	Over	40	7.7
Average	2-12-6	200-300	With	38.7	26.0
	2-12-6	200-300	Side	33.3	44.6
	0-20-10	200	With	33.5	51.4
	0-20-10	200	Side	30.0	55.2
		0		30.7	72.2

[1] Since these plots were on soil relatively low in root rot they are not included in the averages.

Where the same fertilizer treatment was used with and at the side of the seed it is to be noted that in every instance the plants with fertilizer in the furrow were the tallest at this stage. In most instances both were taller than the nearest unfertilized plants. When the percentage of severe root rot is considered it is evident that it was practically always lower in the fertilized plots than in the nearest checks. The adjoining plots of the same treatment usually showed less severe root rot when the fertilizer was applied with the seed than when it was applied at the side of the seed. The application of fertilizer over the seed was not included in all of the comparisons, but where it was so applied the effects were intermediate between the application with and at the side of the seed. There are indications that the nitrogenous fertilizer was more effective than the nonnitrogenous when the 200-pound rate of 2–12–6 and 0–20–10 are compared. This differential effect confirms the observations of Haenseler (*4*, Rept. 56). The fact that, when relatively low rates are used, the benefits are greatest when the fertilizer is immediately available to the plant does not seem to have been emphasized previously. Obviously when the fertilization program requires 1,000 to 2,000 pounds per acre, application with the seed is out of the question because of the danger of seedling injury. With 300-pound rates of formulas relatively low in nitrogen under Wisconsin conditions, however, the application of fertilizer with the seed has not been observed to be

injurious. In fact, better stands are secured more often on the loams and silt loams where moderate applications are applied with the seed.

The appearance of fertilized and unfertilized plants removed from the soil at blossom time was similar to that already observed in the greenhouse and illustrated in figures 1 and 2. The relative height of vines of plants from plots 3, 4, and 5 is shown in figure 3, while the condition of the root systems with respect to root rot in representative plants from each of these plots is illustrated in figure 4. The striking difference between the severity of root rot in the roots from plants grown with fertilizer in the row and at the side of the row is in accord with the actual counts recorded in table 3.

At the canning stage random samples were again taken from plots 3, 4, and 5. Representative plants from each are shown in figure 5. In these plots as well as in the remaining ones the effect of root rot on yield was obvious and the influence of the type of fertilizer placement on the degree of recovery of plants from the disease was a measurable one. In studies of this sort it is difficult to differentiate the direct effect of fertilizer on plant growth and yield from that of the effect on plant resistance. Some measure of this may be taken in the analysis of disease severity already presented. It was fortunate, however, that the plot design was repeated exactly in four other locations in none of which root-rot damage could be found. It is thus possible to get some measure of the indirect effect of fertilizer on yield through disease control by comparing the yield records at all five locations. These are summarized for each treatment in table 4. For each treatment there were duplicate plots at each location. The data from the non-root-rot plots were treated for analysis of variance and the minimum difference, at the 5-percent level, required for significance between the averages of yields for each treatment at four locations is included in the table.

The effectiveness of various fertilizer placements on the non-root-rot plots is of particular interest. In every treatment, except that of 300 pounds of 2–12–6 on the Knox silt loam, the results show the superiority of applying fertilizer down the same drill spout with the seed. The increased yield secured by applying fertilizer in this manner was especially marked on two of the four soil types, namely, the Carrington silt loam and the Superior clay loam. The average of the plots on the Superior clay loam gave a 21-percent increase in yield of fertilized over the unfertilized, and in the case of the Carrington soil it was 27 percent. Similarly the averages for the plots receiving the side application gave increases of 6 and 17 percent, respectively. The averages for each of the treatments for the four different soil types, as indicated in table 4, show increases ranging from 13.7 to 22.2 percent over the unfertilized where the fertilizer application was made with the seed, whereas when it was applied at the side of or above the seed the increase in no case reached 10 percent.

In general the same relative results were maintained in the root rot soil with the additional important fact that the spread between the "side" and "with" application was of much greater magnitude. The highest percentage increase (248.5 percent) was secured where 300 pounds of 2–12–6 were applied with the seed.

While the percentage of increase in yield in fertilized over unfertilized plots was much greater in the root rot soil than in the non-root-rot soil, the actual yields even on the best plots (300 pounds of

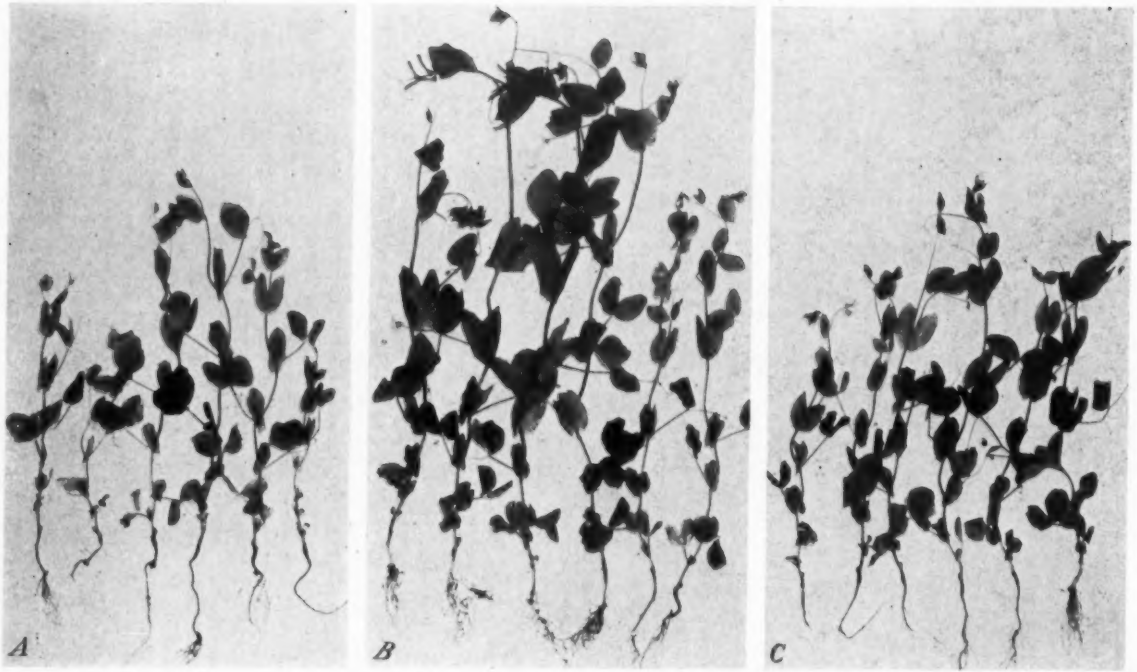

FIGURE 3.—Representative plants taken at the blossom stage from plots 3, 4, and 5 (table 3) of the Marshfield experiment, 1938, on root-rot-infested soil: *A*, No fertilizer; *B*, 300 pounds per acre of 2–12–6 with the seed; *C*, 300 pounds per acre of 2–12–6 at side of seed. Note the relatively greater height of plants from the plot in which fertilizer was applied with the seed. (See fig. 4.)

2–12–6, "with") was much lower than on the plots on which the soil was free of root-rot. It appears that quickly available nutrients placed where root development occurs in its early stage makes it

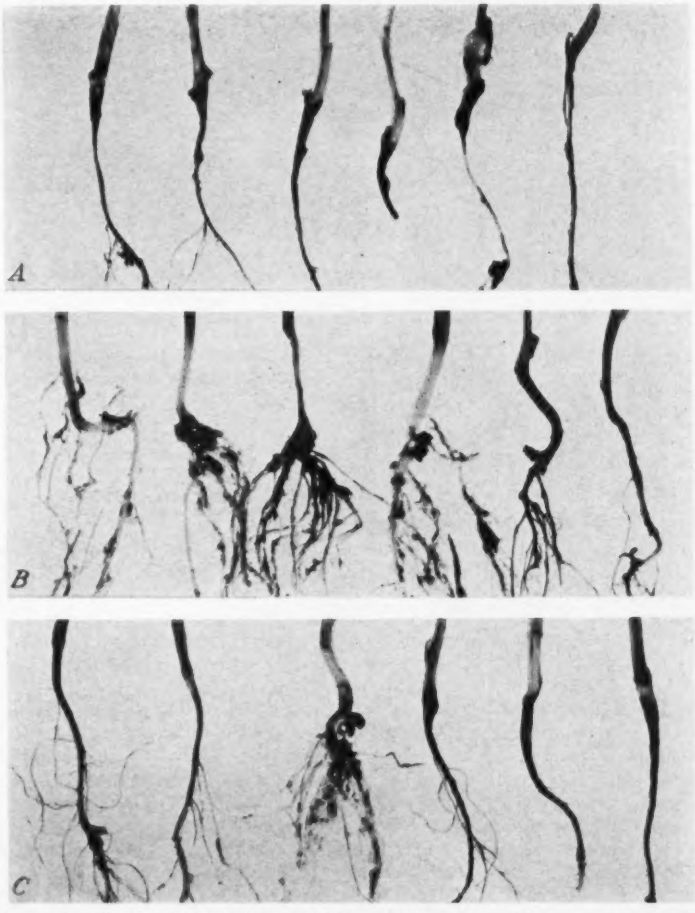

FIGURE 4.—Roots of plants taken at blossom stage from the same plots as those shown in figure 3: *A*, No fertilizer; *B*, 300 pounds of 2–12–6 with the seed; *C*, 300 pounds of 2–12–6 at side of seed. Note that in the unfertilized soil the disease has resulted in decay of most of the fibrous root system as well as the cortical tissue of the lower stem. While the disease is present in the fertilized plants it has been less destructive and many healthy roots are still functional, particularly where the fertilizer was applied with the seed. (See fig. 5.)

possible for the plant to develop greater resistance to the invasion of the root-rot organism. It is evident also that fertilizer placement is an important factor in developing such resistance. Then too, the much greater increase on the plots receiving nitrogen in addi-

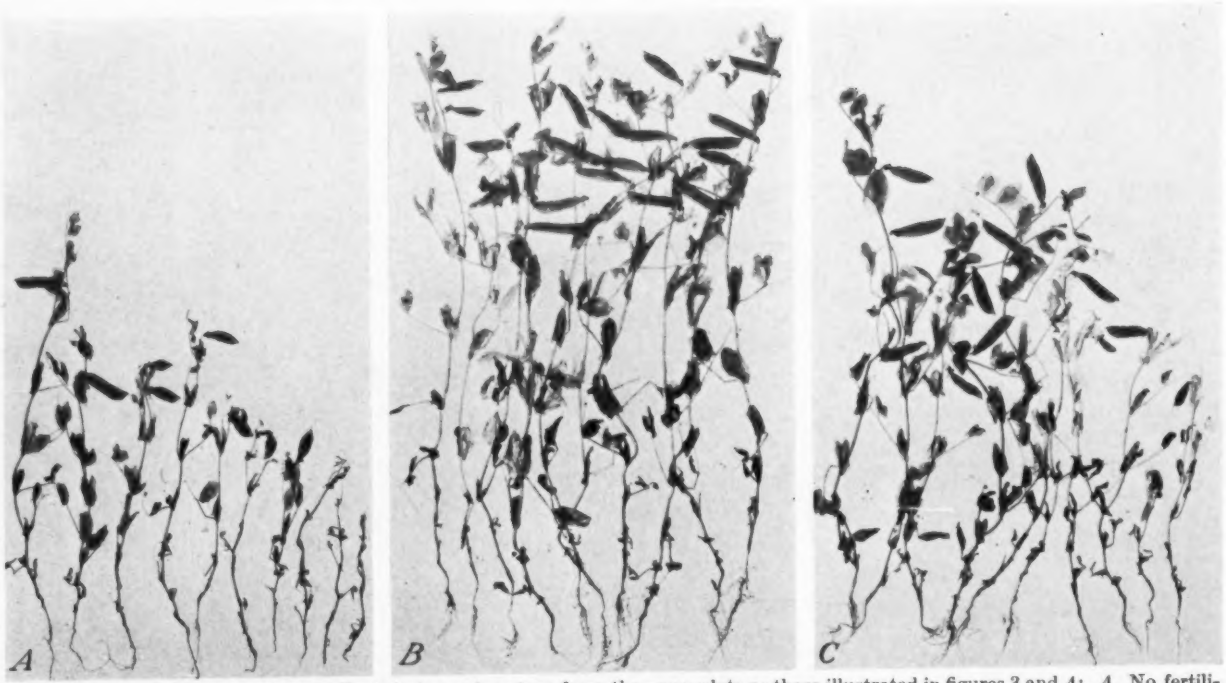

FIGURE 5.—Representative plants collected at canning stage from the same plots as those illustrated in figures 3 and 4: *A*, No fertilizer; *B*, 300 pounds of 2–12–6 with the seed; *C*, 300 pounds of 2–12–6 at side of seed. Note the superiority of the plants which were fertilized by placement with the seed. Compare with the yield data given in table 4.

tion to phosphate and potash brings out the importance of nitrogen in root-rot control as already emphasized at the New Jersey station (*4, Rept. 56*).

TABLE 4.—*The relative yield of peas on root-rot-infested soil (Colby silt loam) and on four other types that were not infested, when various fertilizer treatments were made at the time of sowing, 1938*

Fertilizer treatments			Yield of peas per acre on the indicated soil types at stated locations in Wisconsin							
			Non-root rot soils						Root-rot soil, Colby silt loam near Marshfield	
			Miami silt loam near Columbus[1]	Carrington silt loam near Fall River[1]	Superior clay loam near Oshkosh[2]	Knox silt loam near Durand[1]	Average of non-root rot soils			
Formula	Amount per acre	Placement with relation to seed					Yield	Increase over control	Yield	Increase or decrease over control
	Pounds		*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Percent*	*Pounds*	*Percent*
0-0-0			2,518	2,457	3,040	1,535	2,388		241	
2-12-6	200	With	2,702	3,230	3,390	1,536	2,715	+13.7	509	+111.2
	200	Side	2,484	2,870	2,935	1,440	2,432	+1.8	309	+28.2
	300	With	2,770	2,970	3,820	1,593	2,788	+16.8	840	+248.5
	300	Side	2,508	2,910	3,395	1,632	2,611	+9.3	405	+68.0
	300	Over	2,624	2,790	3,340	1,561	2,579	+8.0	512	+112.4
0-20-10	200	With	[3] 2,948	3,165	3,860	1,702	2,919	+22.2	353	+46.5
	200	Side	2,698	2,830	3,360	1,613	2,625	+9.9	207	−14.1
Difference required for significance at the 5-percent level							230			

[1] Alaska variety used.
[2] Wisconsin Perfection variety used.
[3] Estimated value; 1 degree of freedom lost in error.

SUMMARY

A study was made of the relation of fertilizer treatment to the severity of rot (*Aphanomyces euteiches* Dreschsler) in Colby silt-loam soil.

Experiments in the greenhouse with several varieties of canning peas showed marked control of the disease in three successive crops grown on soil fertilized with one application of 4–16–4 fertilizer at the rate of 500 pounds per acre.

In field experiments conducted on five soil types in five locations in Wisconsin the comparative productiveness of peas and the relative severity of root was studied when fertilizer was applied in various ways. In non-root-rot soils with 200 pounds and 300 pounds per acre, the greatest increases in yield, averaging 13.7 to 22.2 percent above that of the untreated plots, were secured when the fertilizer was applied down the same drill spout with the seed. When the fertilizer was applied about 1½ inches to the side of and at the same level as the seed the increases averaged less than 10 percent.

In general the same relative results were secured in the root-rot-infested soil, with the additional important fact that the percentages of increases in yield were much greater. The difference in the plots where fertilizer was placed at the side of the seed varied from a decrease of 14 percent to an increase of 68 percent over the control, while in the cases where the fertilizer was applied with the seed the increases were from 46 to 248 percent.

It appears that quickly available nutrients placed in amounts of 200 to 300 pounds per acre in the furrow with the seed made it possible for the plants to develop greater resistance to root rot. Much greater reduction of root rot and increases in yield were secured when 2 percent of readily available nitrogen was included in the fertilizer than when it was omitted.

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(5) ———
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RELATION OF TEMPERATURE AND MOISTURE TO NEAR-WILT OF PEA [1]

By WALTER J. VIRGIN, formerly *research assistant*, and J. C. WALKER, *professor of plant pathology, Wisconsin Agricultural Experiment Station*

INTRODUCTION

Practically complete control of the wilt disease (*Fusarium orthoceras* App. and Wr. var. *pisi* Linford), which was formerly very destructive to the pea crop of Wisconsin, eastern Washington, and parts of Maryland, has now been accomplished by the development and general adoption of suitable wilt-resistant varieties. The description of this disease and the causal organism has been given in detail by Linford.[2] More recently another disease of pea, known as near-wilt, has been distinguished in the same areas and even over a wider geographical range. This malady and the causal pathogen (*F. oxysporum* Schlect. f. 8 Snyder) have been described by Snyder and Walker.[3] While the near-wilt disease appears to be much more widespread in the United States than wilt, it has never become so acutely destructive in the regions in which it has become established. In fact it is usually to be found on occasional plants, widely scattered in the field, and only uncommonly does it destroy a majority of the pea plants within a given area. Whether this is due to the relatively poor capacity of the near-wilt organism to establish itself as abundantly and rapidly in favorable soils as does the wilt fungus remains to be determined, but it does appear from field observations that the former organism occurs over a wider range of soil types than the latter.

Symptoms of the two diseases resemble one another in many respects. The foliage becomes yellow with both and the leaflets and stipules of the plants may curve downward and inward, while at high temperatures they may quickly wither from the base of the plant upward. Stunting and rapid loss of turgidity of the wilt disease are less common with near-wilt. On the other hand, unilateral development of symptoms is more common with the latter disease. The pathogen, traveling rather rapidly up the stem, in one or a few vascular bundles, affects leaves and stipules in its path from base to tip. This localized effect commonly extends to one stipule in a pair, to leaflets on one side of the petiole, and even to the portion of the leaf lamina on one side of the midrib. In general the affected vascular system is orange to deep red in color in contrast to the lighter orange characteristic of bundles affected by wilt. This color difference, together with the fact that the near-wilt organism and the discoloration following it travel up the stem often to the growing tip while in wilt they seldom advance above the fifth node, is a very useful means of distinguishing the two diseases.

[1] Received for publication May 22, 1939.

[2] LINFORD, MAURICE B. A FUSARIUM WILT OF PEAS IN WISCONSIN. Wis. Agr. Expt. Sta. Res. Bul. 85 44 pp., illus. 1928.

[3] SNYDER, W. C., and WALKER, J. C. FUSARIUM NEAR-WILT OF PEA. Zentbl. f. Bakt. [etc.] (II) 91: 355-378, illus. 1935. See p. 373.

The purpose of the investigation reported in this paper was to study the effect of certain environal factors, especially temperature and moisture, upon the expression of near-wilt symptoms. This seemed to the writers to be particularly important in view of the attention being given to the possibility of developing near-wilt-resistant varieties suitable for commercial production.

EXPERIMENTAL RESULTS

TEMPERATURE RELATIONS OF THE NEAR-WILT ORGANISM

The relation of temperature to the growth of *Fusarium oxysporum* f. 8 in pure culture was studied in plate cultures of potato-dextrose agar which had been adjusted to pH 6.2. The isolate was from typically diseased plants in the field and its identity was confirmed by

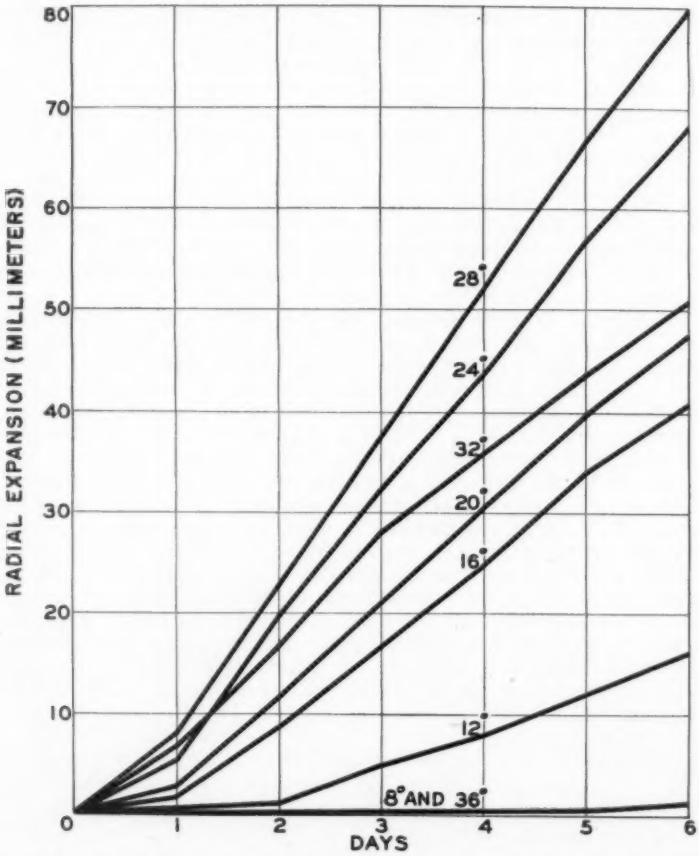

FIGURE 1.—Daily radial expansion of *Fusarium oxysporum* f. 8 on potato-dextrose agar plates incubated at various temperatures.

W. C. Snyder of the University of California. Five inoculated plates were placed in controlled incubators at each of the following temperatures: 4°, 8°, 12°, 16°, 20°, 24°, 28°, 32°, 36° C. After colonies had become established the radial growth of each plate was recorded every 24 hours until the thallus covered the entire plate. No measurable growth occurred at 4°. The average daily radial growth is plotted in a graph for each of the other eight temperatures (fig. 1). Since each of these graphs is practically a straight line it may be assumed that little or no staling material which retarded growth was produced by the fungus. Rates of growth at 8° and 36° were approximately the same. Most rapid radial expansion occurred at 28°. These results agree in general with those reported for other forms of *F. oxysporum*.[4] The maximum, minimum, and optimum temperature for growth as measured by this method came relatively close to coinciding with those of the wilt fungus.[5] It may be pointed out here, however, that whereas the optimum temperature for wilt development is much lower than that for its growth on agar, the data to be presented later in this paper show that the optima are closer in the case of near-wilt.

RELATION OF VARIETY TO DISEASE EXPRESSION

During the course of this investigation a study of varieties of peas (*Pisum sativum* L.) in relation to near-wilt was carried out in both field and greenhouse. It was noted that, generally speaking, the symptoms of near-wilt were slower to appear than those of wilt. Furthermore their development in the case of near-wilt varied with the variety when a number of varieties were grown in the same location. In most cases there was a distinct coincidence under field conditions in southern Wisconsin and in the greenhouse at Madison, Wis., between appearance of near-wilt and the beginning of the blossoming period. Thus early-blossoming varieties showed the disease early while in late-blossoming varieties the appearance of symptoms was delayed. A common example of this is the contrast between the Alaska, a variety which blooms and matures early, and Alderman, a late variety. Under optimum conditions for the disease the plants of Alaska are commonly dead from near-wilt before any signs of disease are shown by the Alderman plants, although the latter eventually succumb. In certain varieties which are very susceptible the plants may wilt before blossoming has occurred. The differential effect of varieties on the progress of the disease has been taken into account, therefore, in the present study of the effect of external environment upon near-wilt.

TEMPERATURE RELATIONS OF THE DISEASE

Soil-temperature experiments were carried out in the greenhouse in Wisconsin tanks. The pathogen was increased on a medium of corn meal and sand which was incorporated with soil that had been collected from an uncultivated wood lot and autoclaved for 5 hours at 15 pounds pressure. Since the volume of inoculum used was very small in relation to that of the soil, a period of 2 months was allowed to elapse during which the mixture was stirred and watered occasionally in order

[4] Goss, R. W. RELATION OF ENVIRONMENT AND OTHER FACTORS TO POTATO WILT CAUSED BY FUSARIUM OXYSPORUM. Nebr. Agr. Expt. Sta. Res. Bul. 23, 84 pp., illus. 1923.
Johnson, James. FUSARIUM WILT OF TOBACCO. Jour. Agr. Res. 20: 515–535, illus. 1921.
[5] See footnote 2.

to permit the organism to become uniformly established. It was then placed in galvanized iron cans which were set into the respective tanks. A quantity of sterilized, uninoculated soil from the same source was placed in other cans to serve as controls. For comparative purposes soil naturally infested with the wilt organism was placed in still other cans.

Five tanks were adjusted to 16°, 20°, 24°, 28°, and 30° C., respectively. In each tank were placed four cans of near-wilt-infested soil, two cans of wilt-infested soil, and 2 cans of uninoculated soil. The moisture content of the soils was approximately 60 percent of their water-holding capacity and the moisture was kept fairly constant by frequent weighings of the cans and replacement of the water lost by evaporation and transpiration.

In each tank two cans of near-wilt soil and one can of uninoculated soil were planted with seeds of Early Kay, a wilt-resistant and somewhat near-wilt-resistant variety of pea, and a similar set was planted with Wilt Resistant Perfection, a wilt-resistant, near-wilt-susceptible variety. The wilt soil (two cans in each tank) was planted with wilt-susceptible Perfection. Ten plants were grown in each can.

Plants were removed and counted as soon as they were permanently wilted and the identity of the disease was confirmed by recovering the respective fungus from each plant. A wilt index or near-wilt index was calculated for each can or duplicate cans by adding the number of days from sowing to wilting for each plant and dividing by the total number of plants, the index being the average number of days for the plants to reach the wilt stage. Thus the more rapid the development of the disease the lower the index. The data from this experiment are given in table 1.

Since the plants in the uninoculated soil all remained healthy they are omitted from the table. The most rapid development of wilt was at 20° C. which is in accord with the report of Linford [6] that the optimum was between 21° and 22°. It is to be seen, however, that the disease development was nearly as rapid at 24°, 28°, and 30° as at 20° while a definite retardation occurred at 16°. In the case of Wilt Resistant Perfection growing on near-wilt soil the most rapid disease development was at 24°, although there was little difference at this temperature from 20° and 28°. Distinct retardation occurred at 16° and 30°. It is evident that the disease was limited more definitely at 30° than was wilt, while at 16° they were both distinctly retarded.

TABLE 1.—*Indices of wilt and near-wilt in Wilt Resistant Perfection (wilt-resistant, near-wilt-susceptible), Perfection (wilt-susceptible), and Early Kay (wilt-resistant, near-wilt-resistant) pea varieties at various soil temperatures*

Variety	Wilt index (days) at soil temperature of—					Near-wilt index (days) at soil temperature of—				
	16° C.	20° C.	24° C.	28° C.	30° C.	16° C.	20° C.	24° C.	28° C.	30° C.
Perfection	[1] 39	25	27	28	[2] 27					
Wilt Resistant Perfection						[3] 64	46	44	46	[4] 55
Early Kay						(5)	(5)	62	63	68

[1] 4 plants alive at the end of the experiment.
[2] 1 plant alive at the end of the experiment.
[3] 2 plants alive at the end of the experiment.
[4] 4 plants dead at the end of the experiment.
[5] No plants dead at the end of the experiment.
[6] 16 plants living, 4 dead at the end of the experiment.

[6] See footnote 2.

In addition to being somewhat more restricted in its optimum soil-temperature range, near-wilt was definitely slower in appearing. Thus in the two strains of Perfection it required, on an average, nearly twice as long for all plants to succumb to near-wilt at 20° to 28° C. as was the case with wilt at the same temperatures. The

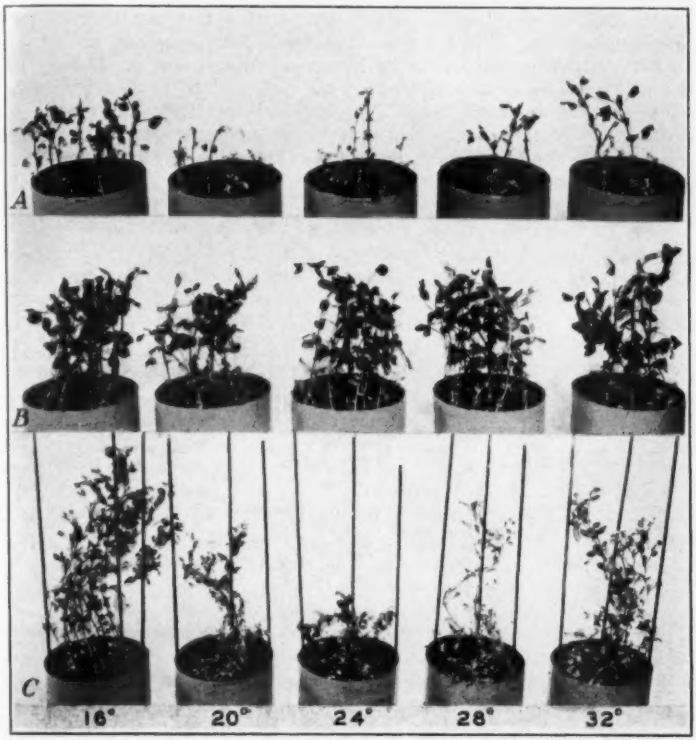

FIGURE 2.—Relation of soil temperature to the development of wilt and near-wilt. *A*, Wilt-susceptible Perfection peas grown in wilt-infested soil for 30 days at the respective constant soil temperatures indicated at the bottom of the figure. *B*, Near-wilt-susceptible Wilt Resistant Perfection plants grown for 30 days in near-wilt-infested soil in parallel series with those in *A*. Note that at this interval the wilt-susceptible plants are all dead or severely diseased at 20°, 24°, and 28° C., while no signs of disease have appeared in the plants on near-wilt soil. *C*, The same series as *B* which has been continued at the respective temperatures for 22 days longer. Note that near-wilt has now developed to a severe degree at 20°, 24°, and 28°, showing that it requires much longer to develop than does wilt, even in optimum environment.

data from Early Kay showed the effect of plant resistance on lengthening the time required for plants to succumb. They showed also that at the higher temperatures the disease eventually affected all plants, even of a variety which shows marked resistance under field conditions. Certain of the plants in this experiment are illustrated in figure 2.

The same test was applied with the inoculated near-wilt soil in a second experiment. Only the most favorable temperatures, 20°, 24°, and 28° C., were included. The two varieties used were Alaska and Horal. The latter, being a late-blooming variety, had shown a longer incubation period in the field and definite evidence of resistance. In a third test Alaska and Wilt Resistant Perfection were run at 16°, 20°, and 24° in naturally-infested near-wilt soil from a field near Winneconne, Wis. The results of these trials are given in table 2. The near-wilt index tended to be lower in the naturally-infested than in the artificially inoculated soil. This is found to be the case when the two Alaska tests in table 2 and the Wilt Resistant Perfection tests in tables 1 and 2 are compared. It may be due to a heavier infection or to the supplementary effect of other organisms which are pathogenic on pea roots, in the naturally infested soil. The same inoculated soil was used several months later for the next experiment (table 3), in which it will be noted that the near-wilt index is about the same for Wilt Resistant Perfection as that in the naturally infested soil of the last experiment. Whether this change is due to the increase in the amount of near-wilt inoculum or to the influence of other organisms with which contamination had occurred cannot be stated.

TABLE 2.—*Near-wilt indices in certain pea varieties in artificially inoculated and in naturally infested soil*

Type of soil infestation	Variety of pea	Near-wilt index [1] at soil temperature of—			
		16° C.	20° C.	24° C.	28° C.
Artificially inoculated	Horal	.....	82	80	74
	Alaska	.....	46	42	49
Naturally infested	Alaska	[2]	30	36	.....
	Wilt Resistant Perfection	[3]	46	38	.....

[1] The near-wilt index in each case is based on 10 plants.
[2] At 60 days only 1 plant had wilted.
[3] At 60 days only 2 plants had wilted.

It is to be noted that, in accord with what has been pointed out in the previous section, the index was higher in Wilt Resistant Perfection, a late-blooming variety, than in Alaska, an early-blooming variety. This difference was only slight, however, at the optimum. Alaska and Wilt Resistant Perfection are regarded as very susceptible to near-wilt. The comparison between Alaska and Horal brings out again the striking difference in index between an early susceptible and a late resistant variety. Horal is about the same in season as Wilt Resistant Perfection but the near-wilt index of the former is nearly twice that of the latter, discounting the slight discrepancy to be attributed to the difference between inoculated and naturally infested soil. Thus the variety usually highly resistant in the field shows considerable resistance in the greenhouse, but eventually succumbs.

The same artificially inoculated soil used in the above experiments was employed in a study of the relation of air temperature to the development of near-wilt. Three soil temperatures were used—16°, 22°, and 28° C.—at each of three air temperatures—16°, 20 to 22°, and 28°. Wilt Resistant Perfection was planted in three cans at each soil temperature in each air temperature, the total number of plants

in each three-can group averaging about 35. The near-wilt indices secured in this experiment are recorded in table 3. The optimum soil temperature was 28° regardless of the air temperature to which the tops were exposed. At any given soil temperature the differences in rate of disease development between the three air temperatures was not great although the smallest index in each case was at 20°–22°.

TABLE 3.—*Near-wilt indices in Wilt Resistant Perfection peas grown in artificially inoculated soil at 3 soil temperatures in each of 3 air temperatures for 57 days*

Air temperature (° C.)	Near-wilt index at soil temperature of—		
	16° C.	22° C.	28° C.
16°	[1] 51	38	28
20°–22°	[2] 47	34	25
28°	[3] 52	35	27

[1] Based on 8 diseased plants from a total of 37.
[2] Based on 25 diseased plants from a total of 48.
[3] Based on 13 diseased plants from a total of 42.

It may be concluded from these experiments that the soil temperature is much more influential upon near-wilt than is air temperature. While an exact soil-temperature optimum cannot be defined it is evident that constant soil temperatures of 24° to 28° C. usually result in the most rapid development of the disease. Thus the optimum for near-wilt may be regarded as about 5° higher than that for wilt. Temperatures around 16° greatly retard near-wilt regardless of the air temperature. On the other hand, an air temperature of 16° does not appreciably affect the progress of the disease provided the soil temperature is maintained near the optimum. These statements apply particularly to the near-wilt susceptible varieties such as Alaska and Wilt Resistant Perfection. In the second experiment (table 2) there is an indication that the resistant variety, Horal, has a higher optimum than the susceptible variety, Alaska. These studies should be continued with resistant varieties.

RELATION OF SOIL MOISTURE TO THE DISEASE

Soil-moisture studies were conducted in Wisconsin soil-temperature tanks where the temperature variable could be eliminated by running all tanks at 24° C. in a common air temperature of 21° to 22°. Three soil types were used—two infested with near-wilt, one not infested. The infested soils were: (1) A sandy loam from a naturally infested field at Winneconne, already mentioned in connection with the soil-temperature experiments; and (2) a black silt loam which had been artificially inoculated. The noninfested soil was also a silt loam, but was lower in organic matter than the inoculated soil. It was sterilized before use. These soils were each made up into three groups designated as dry, medium-moist, and wet. The water-holding capacity of each soil and the actual moisture content of each group were determined. The percentage of water-holding capacity for each group is given in table 4.

The data from two series, including two susceptible (Alaska and Wilt Resistant Perfection) and two resistant (Kay and Early Kay) varieties are given in table 5. Inasmuch as no disease developed in the noninfested soil no data therefrom are included. The best growth in this soil occurred in the medium-moist lot. There was distinct retardation in growth in the dry soil. In the wet soil, yellowing of the lower leaves was pronounced.

TABLE 4.—*The water-holding capacity of the lots of soil used in the study of the relation of soil moisture to near-wilt*

Description of soil	Moisture as water-holding capacity in—		
	Dry group	Medium-moist group	Wet group
	Percent	*Percent*	*Percent*
Naturally infested sandy loam	25	47	67
Artificially inoculated silt loam	32	69	84
Sterilized uninoculated silt loam	30	55	75

TABLE 5.—*The relation of soil moisture to the development of near-wilt in pea*

Description of soil	Variety of pea	Series No.	Dry soil		Medium-moist soil		Wet soil	
			Plants used [1]	Near-wilt index	Plants used [1]	Near-wilt index	Plants used [1]	Near-wilt index
			Number		*Number*		*Number*	
Inoculated silt loam	Wilt Resistant Perfection	1	19	64	13	68	27	
		2	12	54	17	50	17	
	Alaska	1	41	51	51	47	54	
		2	20	51	20	47	20	
	Early Kay	2	19/6	55	19/8	61	20/4	
	Kay	2	19/8	67	19/0	.....	20/14	
Naturally infested sandy loam	Wilt Resistant Perfection	1	31	54	37	51	14	
		2	17	44	18	46	19	
	Alaska	1	31	41	56	41	42	
		2	17	41	20	40	17	
	Early Kay	2	16	82	14	39	19	
	Kay	2	20	80	17	54	14/12	

[1] In those cases in which all plants did not wilt permanently before the close of the experiment a fraction is given of which the numerator is the total number of plants and the denominator is the number of plants that did wilt permanently; the index is based on the data from the latter group only.

It will be seen from the data that in the case of Wilt Resistant Perfection and Alaska there was little difference between the indices in dry and medium-moist soil, while those in the wet soil were consistently the lowest. In the naturally infested soil disease development was generally more rapid, but the same relation prevailed between the dry, medium, and wet levels. Whether the greater rate of disease advance in the naturally infested soil is due to the supplementary effect of other organisms is not known, but it is recalled that the same relation occurred between artificially inoculated and naturally infested soil in the soil-temperature studies.

When the resistant varieties, Kay and Early Kay, are considered the soil-moisture effects are not so clear. The fact that disease development was so slow that usually only a small portion of the plants entered

into the index may account in part for the lack of conformity. In the naturally infested soil, where practically all of the plants wilted, the index of both resistant varieties was distinctly lower in the medium-moist soil than in either the dry or the wet soil.

DISCUSSION

Although the wilt and near-wilt diseases have closely related causal organisms and certain symptoms in common, they nevertheless are distinct in several respects. Perhaps the greatest important difference is that pea plants and varieties fall into two discontinuous groups insofar as resistance and susceptibility to wilt are concerned, while in the case of near-wilt, the distinction between susceptible and resistant types is not so clear. Under very favorable conditions for near-wilt all plants of resistant forms slowly but eventually succumb. The effect of the host plant itself upon the development of the disease is more striking in near-wilt. This is shown in the correlation between rate of disease development and the rate of blooming of the host. It is again shown in the increase in incubation period in proportion to host resistance. These varietal differences usually held at various soil temperatures and moistures.

The relative slowness of near-wilt development as compared with wilt development, regardless of variety, is outstanding. It is probably as important a factor as any in determining the fact that although the near-wilt organism is more widespread it is generally less destructive than wilt.

The fact that the optimum soil temperature for near-wilt is somewhat higher than that for wilt is not likely to be very important since near-wilt is nearly as destructive at 20° and 28° C. as at 24° and is limited at 16° to about the same degree as wilt. While susceptible varieties succumb most rapidly in wet soils the progress in moderately moist and dry soils is sufficiently rapid to indicate relatively little retardation in dry seasons.

Although the effects of temperature and moisture were quite consistent and clear-cut when susceptible varieties were used, it is important to note that they did not always coincide with those secured with resistant forms. These discrepancies warrant further study on these and other resistant varieties and need to be considered in the evaluation of plants in improvement of the pea for resistance to near-wilt.

SUMMARY

The investigations comprise a study of the temperature and soil-moisture relations of the near-wilt fungus (*Fusarium oxysporum* Schlect. f. 8 Snyder) in relation to the pea plant.

On potato-dextrose agar the most rapid radial expansion of the organism occurred at 28° C. and the upper and lower limits for growth were somewhat above 36° and below 8°.

The near-wilt disease develops more slowly in a favorable environment than does wilt (*F. orthoceras* App. and Wr. var. *pisi* Linford).

Varieties differ in the rate at which near-wilt develops, the disease appearing more slowly as a rule in late-blossoming forms than in early-blossoming ones.

The optimum soil temperature for near-wilt is about 24° to 28° C. and it is thus about 5° higher than that for wilt. The disease develops readily, however, at temperatures as low as 20°; at 16° it is distinctly retarded. Air temperature has relatively little influence upon the disease.

In near-wilt-susceptible varieties there is little difference in the rate of wilting in dry and medium-moist soil, but it is consistently more rapid in moist soil. In the resistant varieties used the wilting is most rapid in medium-moist soil. The rate in all soils is sufficiently rapid, however, to indicate little retardation of the disease in dry seasons.

PHYSIOLOGICAL STUDIES OF LEMONS IN STORAGE[1]

By ERSTON V. MILLER, *physiologist*, and HAROLD A. SCHOMER, *assistant physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

It has long been known that the practice of storing lemons (*Citrus limonia* Osbeck) at a temperature of 40° F. and lower is conducive to the development of physiological disorders such as membranous stain and pitting. However, since higher temperatures are undesirable because they stimulate the growth of fungus parasites, many attempts have been made to treat the fruit in such a manner that the lower temperatures may be employed. A study of the metabolism of these fruits when subjected to various storage temperatures should yield information regarding fundamental causes of the physiological disorders.

Brooks and McColloch[3] reported that pitting of lemons did not occur in storage at 60° F. and was seldom serious at 50° but was the chief limiting factor at lower temperatures if the storage period was extended beyond 4 weeks. They found that pitting was much worse at 32° and 36° than at 40°. Membranous stain of lemons occurred at 32°, 36°, 40°, 50°, and 60° in the following relative degree of severity, 1, 12, 22, 8, and 2, respectively, with usually only slight traces at 32° but with 75 to 100 percent of the fruits affected at 40°. These investigators were able to reduce the percentage of both disorders by a prestorage treatment with carbon dioxide or by waxing the fruit.

MATERIAL AND METHODS

Green California lemons were shipped under ventilation in carlot shipments from Los Angeles, Calif., to New York City and were forwarded to Washington, D. C., by ordinary express. Temperatures of 32°, 36°, 40°, 50°, and 60° F. were maintained in the cold-storage rooms at Arlington Experiment Farm, Arlington, Va. Enough lemons were stored at each temperature to permit each sample to consist of 30 fruits.

For chemical analyses the whole peel (flavedo and albedo) was separated from the pulp. Seeds were removed from the segments of pulp. For sugar and acid determinations, both peel and pulp were ground in a food chopper. Details of these analyses were identical with those described in a previous article.[4] Glycoside content of the peel was determined by both Harvey's method[5] and that of Darwin

[1] Received for publication February 18, 1939.

[2] The writers are indebted to Charles Brooks and L. P. McColloch, of this Division, for making samples available for chemical analysis and for supplying the pathological data.

[3] BROOKS, CHARLES, and MCCOLLOCH, LACY P. SOME EFFECTS OF STORAGE CONDITIONS ON CERTAIN DISEASES OF LEMONS. Jour. Agr. Research 55: 795–809, illus. 1937.

[4] MILLER, ERSTON V., and DOWD, OSCAR J. EFFECT OF CARBON DIOXIDE ON THE CARBOHYDRATES AND ACIDITY OF FRUITS AND VEGETABLES IN STORAGE. Jour. Agr. Research 53: 1–17, illus. 1936.

[5] HARVEY, E. M. PHLORIDZIN. I. THE SIGNIFICANCE OF PHLORIDZIN IN APPLE AND PEAR TISSUE. II. THE HYDROLYSIS AND ESTIMATION OF PHLORIDZIN. Oreg. Agr. Expt. Sta. Bull. 215, 23 pp. 1925.

and Acton.[6] The latter method proved more satisfactory, and only the results of this method are presented.

Reductase of the peel was determined as follows: 25 gm. of the ground peel was macerated by means of sand and a mortar and pestle and extracted with 100 ml. of water for 15 minutes. One milliliter of this filtered extract was added to 3 ml. of potassium permanganate ($KMnO_4$) (1.33 gm. per liter) and 2 ml. of N/10 oxalic acid. These solutions were held at 68° F. until they attained an amber color, and the time in minutes required to reach this end point was recorded.

For acetaldehyde determinations the pulp was not ground as for the sugar analysis. The details of the method have already been published.[7] Acetaldehyde determinations on citrus peel were vitiated by the presence of interfering substances and were not considered reliable.

RESULTS

SUGAR

In tables 1 and 2 will be found the values for the sugar content of the lemon peel before, during, and after the storage period. With but one exception the results show a decrease in reducing sugar during storage for all temperatures. Usually the higher the storage temperature the greater the loss of reducing sugar. In the first experiment for 1934 (table 1) the peel of the fruits at 50° F. lost 23 percent of its reducing sugar in 13 weeks; in the second experiment of the same year the peel of the fruits at 50° lost 33 percent of its original reducing sugar in 11 weeks. The 32° lots in the first experiment showed a slight gain, while those in the second experiment had lost 5.7 percent at the end of the storage period. In 1935 (table 2) the peel of the fruit stored at 50° lost 26.5 percent of its original reducing sugar in 15 weeks; the 32° lot lost 15.7 percent of its reducing sugar during the same time. For some unknown reason the lots at 36° and 40° in 1935 lost more sugar than other lots. These losses in reducing sugar are also reflected in the total-sugar values. Sucrose occurs in relatively small quantities in both the peel and pulp of lemons, and it is difficult to interpret the values for this substance; it sometimes increased and sometimes decreased, but with no apparent consistency.

In 1935 the pulp, as well as the peel, was analyzed for sugar (table 2). The results for the pulp were quite similar to those for the peel. Reducing sugar and total sugar diminished in quantity during the storage period, and the decrease was greatest at the higher temperatures. There was still less sucrose in the pulp than in the peel.

In all these results there is nothing to indicate that the changes in sugar content might be related to pitting of the peel. The greater loss of reducing sugar at 36° and 40° F. in the 1935 experiments suggests a relationship with pitting, but these results had not been observed in the 40° lots in 1934 and can hardly be considered as significant.

[6] DARWIN, FRANCIS, and ACTON, E. HAMILTON. PRACTICAL PHYSIOLOGY OF PLANTS. Ed. 3, 340 pp., illus. Cambridge. 1901. (See p. 285.)

[7] MILLER, ERSTON V. DISTRIBUTION OF ACETALDEHYDE AND ALCOHOL IN THE APPLE FRUIT. Jour. Agr. Research 53: 49-55. 1936.

TABLE 1.—*Sugar content* [1] *of peel of lemons stored at three temperatures, 1934*

MARCH TO JUNE (EXPERIMENT 1)

Storage temperature (° F.)	Reducing sugar			Sucrose			Total sugar		
	At beginning of storage period	After storage for—		At beginning of storage period	After storage for—		At beginning of storage period	After storage for—	
		7 weeks	13 weeks		7 weeks	13 weeks		7 weeks	13 weeks
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
50	4.77	3.93	3.65	1.09	0.84	0.64	5.86	4.77	4.29
40	4.77	4.29	4.11	1.09	1.05	1.12	5.86	5.34	5.23
32	4.77	5.05	5.09	1.09	1.10	1.57	5.86	6.15	6.66

JUNE TO SEPTEMBER (EXPERIMENT 2)

		4 weeks	11 weeks		4 weeks	11 weeks		4 weeks	11 weeks
50	4.01	3.35	2.68	0.61	0.70	0.72	4.62	4.05	3.40
40	4.01	3.80	2.76	.61	.89	.79	4.62	4.69	3.55
32	4.01	3.74	3.78	.61	.82	.80	4.62	4.56	4.58

[1] Percentage of fresh weight.

TABLE 2.—*Sugar content* [1] *of peel and pulp of lemons stored at various temperatures, January to May 1935*

Storage temperature (°F.)	Peel									Pulp								
	Reducing sugar			Sucrose			Total sugar			Reducing sugar			Sucrose			Total sugar		
	At beginning of storage period	After storage for—		At beginning of storage period	After storage for—		At beginning of storage period	After storage for—		At beginning of storage period	After storage for—		At beginning of storage period	After storage for—		At beginning of storage period	After storage for—	
		9 weeks	15 weeks		9 weeks	15 weeks		9 weeks	15 weeks		9 weeks	15 weeks		9 weeks	15 weeks		9 weeks	15 weeks
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
60	4.15	3.58	2.98	0.58	0.64	0.71	4.73	4.22	3.69	1.92	1.53	1.32	0.09	0.26	0.10	2.01	1.79	1.42
50	4.15	3.99	3.05	.58	.64	.52	4.73	4.63	3.57	1.92	1.91	1.43	.09	.21	.36	2.01	2.12	1.79
40	4.15	3.28	2.86	.58	.74	.66	4.73	4.02	3.52	1.92	1.41	1.49	.09	.34	.32	2.01	1.75	1.81
36	4.15	3.35	2.67	.58	.77	.53	4.73	4.12	3.20	1.92	1.60	1.70	.09	.43	.12	2.01	2.03	1.82
32	4.15	3.78	3.50	.58	.79	.77	4.73	4.57	4.27	1.92	1.63	1.67	.09	.38	.24	2.01	2.01	1.91

[1] Percentage of fresh weight.

GLYCOSIDES AND TOTAL ACIDITY

The glycoside content of the peel is shown in table 3. These substances are present in relatively small amounts in fruits. The glycoside content of the peel at the end of 4 weeks' storage differed little from the original amount. The fluctuations at 40° and 50° F. are doubtless due to sampling errors. At 32° the quantity increased from 0.74 to 0.90 percent in 4 weeks. On the other hand, the 11 weeks' sample showed definite increases in glycosides. From the original sample, which had a glycoside content of 0.74 percent, the lots stored at 50°, 40°, and 32° showed an increase to 1.12, 1.26, and 1.08 percent, respectively.

The results for total acidity of the peel were somewhat similar to those for glycosides (table 3). The total acidity at the end of 7 weeks' storage showed no increase in the 40° and 50° F. lots and only a slight increase at 32°. At the end of 13 weeks there was a consistent increase in total acidity of the peel. The original sample at the beginning of storage contained 0.16 percent of acid. At the end of the storage period the 50°, 40°, and 32° lots contained 0.23, 0.18, and 0.21 percent of total acid, respectively. There was no apparent relation between total acidity and pitting or between glycosides and pitting.

TABLE 3.—*Glycoside content* [1] *and total acidity* [2] *of peel of lemons stored at three temperatures*

Storage temperature (°F.)	Glycosides			Total acidity		
	At beginning of storage period	After storage for—		At beginning of storage period	After storage for—	
		4 weeks	11 weeks		7 weeks	13 weeks
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
50	0.74	0.66	1.12	0.16	0.14	0.23
40	.74	.70	1.26	.16	.15	.18
32	.74	.90	1.08	.16	.18	.21

[1] Percentage of fresh weight.
[2] Percentage of acid as citric in fresh peel.

ACETALDEHYDE

The acetaldehyde content of the lemon flesh at various storage periods is presented in table 4. Inasmuch as the quantity evolved from the same variety of fruit may vary with different pickings and with different years, the results of all four experiments are included in the table. Acetaldehyde was highest in the last samples taken, if the storage period extended to 13 or 15 weeks, because many of the fruits had been held until internal break-down had begun. The amount of acetaldehyde at this period ranged from 0 to 1.3 mg. per 100 gm. of fresh material. A slight amount had accumulated in the midstorage sample. In several instances there was slightly more acetaldehyde in the 40° F. lots than in others, but this was not consistent. In fact, there was nothing to indicate that physiological disorders, like pitting and membranous stain, could be traceable to accumulation of acetaldehyde at these lower temperatures.

TABLE 4.—*Acetaldehyde content* [1] *of pulp of lemons stored at various temperatures, 1934 and 1935*

1934

Storage temperature (°F.)	Acetaldehyde		
	At beginning of storage period	After storage for—	
		7 weeks	13 weeks
	Milligrams	*Milligrams*	*Milligrams*
50	0.0	0.7	0.9
40	0	.7	1.0
32	0	.9	0

1935 (EXPERIMENT 1)

		9 weeks	15 weeks
60	0.0	0.2	0.0
50	0	.2	.2
40	0	.3	.6
36	0	.4	1.3
32	0	.1	.9

1935 (EXPERIMENT 2)

		6 weeks	8 weeks
60	0.0	0.0	0.0
50	0	0	0
40	0	.1	.2
36	0	0	.3
32	0	0	.3

[1] Milligrams per 100 gm. of fresh pulp.

REDUCTASE

The results for reductase determinations are presented in table 5. Reductase is expressed as time in minutes required for an aqueous extract of the peel to reduce a standard potassium permanganate solution. The reductase value for the original or prestorage samples ranged from 3.00 to 9.67 minutes. Subsequent studies indicated that this variation may be due to difference in maturity of fruit. The reductase values are therefore relative and must be compared only with lots of fruit in the same experiment. In all four of the experiments reported, the time required for reduction of the potassium permanganate was greatest for the fruits stored at temperatures of 40° F. or lower. Thus, in the midstorage samples of the first experiments for 1934 the value for the 40° lot exceeds that for the 50° lot by 1.83 minutes, and that for the 32° lot by 1.15 minutes. In the second experiment the excess of reducing time of the midstorage 40° lot over the 50° lot was 7.22 minutes, and over the 32° lot it was 4.15 minutes.

TABLE 5.—*Reductase activity* [1] *of peel of lemons stored at various temperatures, 1934 and 1935*

Storage temperature (°F.)	Reductase activity			Disease record at end of storage	
	At beginning of storage period	After storage for—		Area affected by pitting	Fruit showing membranous stain
		7 weeks	13 weeks		
	Minutes	*Minutes*	*Minutes*	*Percent*	*Percent*
1934 (EXPERIMENT 1)					
50	3.00	4.12	3.79		31.4
40	3.00	5.95	5.16		99.9
32	3.00	4.80	4.74		6.6
1934 (EXPERIMENT 2)					
		4 weeks	11 weeks		
50	6.78	6.86	5.50		88.2
40	6.78	14.08	5.97		91.3
32	6.78	9.93	7.20		40.0
1935 (EXPERIMENT 1)					
		9 weeks	15 weeks		
60	9.67	5.00	3.27	29.3	19.4
50	9.67	4.33	4.55	11.7	73.3
40	9.67	7.33	8.75	40.0	93.3
36	9.67	8.29	9.25	76.5	83.8
32	9.67	9.95	8.52	60.0	6.7
1935 (EXPERIMENT 2)					
		6 weeks	8 weeks		
60	7.66	6.37	6.25	0	20.0
50	7.66	6.37	5.75	0	83.3
40	7.66	6.66	7.33	12.6	96.3
36	7.66	8.91	7.33	22.3	30.9
32	7.66	7.65	7.87	5.7	6.9

[1] Expressed as time in minutes required by aqueous extract of peel to reduce potassium permanganate.

In the 1935 experiment (table 5) a similar relationship held. It may be shown in a different manner. In the midstorage sample of the first experiment the average value for the temperatures 50° and 60° F. was 4.67, while that for the lower temperatures (40°, 36°, and 32°) was 8.52, almost twice as high. In the second experiment of the same year the average value for the temperatures 60° and 50° was 6.37 and that for the lower temperatures was 7.74. Since reductase activity is indicated as the time required to reduce the potassium permanganate solution, a high value in the table indicates a low reductase value. Therefore, the lots stored at temperatures that produced the most pitting (40°, 36°, and 32°) showed the lowest reductase activity. While there is no direct correlation between these values and pitting in lemons, the temperatures that produced the most pitting in storage (40°, 36°, and 32°) showed the lowest reductase activity.

DISCUSSION

The sugar analyses made of the peel of lemons during storage do not suggest any abnormality in the metabolism of the fruit at any tem-

perature studied. One would expect the reducing sugar to be consumed during the respiratory processes. The greater loss at higher temperatures is also natural. Similarly, the acetaldehyde determinations do not indicate any abnormality in the fruit. In these experiments the fruits yielded varying amounts of acetaldehyde after they had been in storage awhile. No unusual quantities were found in fruit stored at 36° or 40° F., the temperatures so conducive to membranous stain in lemons. Whatever the factors that cause these physiological disorders may be, they apparently do not affect sugar consumption or the formation of acetaldehyde.

No additional information on physiological disturbance was obtained from the acid and glycoside values. The slight increase in total acid as storage was continued may be the result of dehydration. The glycoside content of the peel shows a much greater increase. This may be a natural aging process. Again, there is nothing in these results to indicate that the fruit at the lower temperatures will show a higher percentage of physiological disorders.

A slightly different result is obtained from the reductase studies. The reductase activity in the peel of the fruit stored at 32°, 36°, and 40° F. was always lower than in that stored at 50° and 60°. The first three temperatures are known to be most conducive to the production of pitting in lemons. Apparently some substance in the peel is oxidized more rapidly at these lower temperatures and is therefore not oxidized by the potassium permanganate solutions. The fact that sometimes the pits assume a dark appearance also suggests the action of oxidases. Reducing sugar and acetaldehyde might account for some part in the reduction of potassium permanganate, but the reaction is certainly enzymatic, because boiling the peel destroys its ability to reduce potassium permanganate at different rates.

SUMMARY

Biochemical studies were made of lemons stored at 32°, 36°, 40°, 50°, and 60° F.

The peel was analyzed for sugar, glycosides, acids, and reductase activity before, during, and after storage. The flesh was analyzed for sugar and acetaldehyde.

Reducing sugar and total sugar in both peel and flesh diminished in quantity during storage of the fruit. These tissues contained only slight amounts of sucrose.

Both total acids and glycosides in the peel increased during storage.

Varying amounts of acetaldehyde were found in the flesh at the time of the midstorage and final sampling dates.

No relation was found between any of the above-mentioned substances and the development of pitting and membranous stain in the fruit.

Reductase activity of the peel, as measured by the rate of reduction of potassium permanganate solutions, was consistently lower for the samples stored at 32°, 36°, and 40° F. than for those stored at 50° and 60°. Inasmuch as the lower temperatures (32°, 36°, 40°) are most conducive to development of pitting of lemons in storage, it is suggested that oxidizing enzymes may play a role in the development of this disorder.

INFECTION AND REINFECTION EXPERIMENTS WITH BANG'S DISEASE[1]

By B. H. EDGINGTON, *animal pathologist*, and C. R. DONHAM, *associate in animal industry, Ohio Agricultural Experiment Station*

INTRODUCTION

The experiments reported here are concerned with: (1) The effect of time of breeding and stage of gestation on the agglutinin response of animals exposed to *Brucella abortus;* (2) the channels of entrance of the bacteria into the animal body; and (3) the result of reinfection of animals.

The course of Bang's disease in female cattle following natural exposure is generally recognized as being extremely complicated. The results that follow infection depend to a large extent on the status of the animal at the time infection occurs. The term "status of the animal" as used in this paper refers to such variables as age, puberty, the presence or absence of pregnancy, and the duration of gestation.

The first point of interest in these experiments is associated with the variable results obtained with six groups of cattle having different breeding histories at the time of their experimental infection. Observations similar to some of these have been recorded by other investigators; however, the relationship of these results to the status of the animal at the time of infection appears not to have received particular emphasis.

The second phase of these experiments pertains to the channels of entrance of *Brucella abortus* organisms into the animal body. Numerous investigations have shown that there are various avenues through which the bacteria may gain entrance to the animal body; namely, the digestive tract, the conjunctival membrane, the teat canal, the vagina, the unbroken skin, and by subcutaneous or intravenous injection.

There still remains some question as to whether or not the channel of entrance has an effect on the reaction of the animal following infection. In other words, do the reactions of particular tissues influence the course of the disease?

In these experiments some infections were per vagina, some per conjunctiva, and some per os.

In the vaginal exposures the vagina may or may not have been the sole channel of entrance of the infection (as will be discussed later), nevertheless the results warrant consideration.

The third phase of the work relates to reinfection and is one concerning which data are relatively meager. Vaccination experiments, in which animals have been inoculated subcutaneously with organisms of various degrees of pathogenicity, have frequently included reinfections, but the data usually have not been analyzed in terms of reinfection experiments.

There is considerable clinical evidence suggesting that many of the symptoms attending this disease are associated with repeated rein-

[1] Received for publication April 19, 1939.

fections of the animal rather than with a single, initial infection. However, so far as the writers know, but few controlled experiments have been undertaken to verify such clinical observations. The experiments reported here appear to have a bearing on this phase of the course of Bang's disease under natural conditions.

EXPERIMENTAL PROCEDURE

Infections were produced in the following ways: (1) By introducing *Brucella abortus* organisms into the vagina; (2) by instilling the organisms into the conjunctival sac; and (3) by feeding grain mixed with suspensions of the bacteria. Several different strains of *Br. abortus* isolated from cattle were grown for 48 to 96 hours on the usual pork- or liver-agar media. The growths were washed from the media with physiologic salt solution and the bacterial suspensions standardized to 10 times tube No. 2 of the McFarland nephelometer. Vaginal exposures were made by slowly injecting 20 to 40 cc. of the fresh bacterial suspensions into the vagina, eye exposures by instillation of the suspension into the lachrymal sac, and oral exposure by the daily feeding on 3 successive days of a culture from one tube of medium mixed with the grain for each cow. Eighteen of the cattle inoculated through the vagina in the earlier tests were kept muzzled, except at feeding and watering time, for from 6 to 8 weeks after inoculation. This was done to minimize the chances of the bacteria gaining entrance to the alimentary canal. During the time that the animals were muzzled they were housed constantly in separate stanchions with rails between each animal. The muzzling was discontinued in later experiments, after it seemed apparent that animals regularly became infected by this method of exposure. (See discussion of group 1.)

Agglutination blood titers were determined at approximately 1-month intervals beginning at the time of exposure of the animal. Antigen for the tests was prepared essentially in accordance with the method recommended by the United States Live Stock Sanitary Association.[2]

All the animals were free from Bang's disease at the time they were placed in the experiments, as judged by clinical histories and repeated negative agglutination tests. Six different groups of cattle were used in the experiment, viz: (1) Heifers exposed to *Brucella abortus* during difference stages of pregnancy; (2) heifers exposed prior to pregnancy; (3) heifers exposed prior to breeding and reexposed subsequent to pregnancy; (4) heifers exposed prior to and reexposed subsequent to breeding that did not result in pregnancy; (5) heifers exposed before their first pregnancy and reexposed during their second pregnancy; and (6) cows exposed prior to and subsequent to pregnancy.

EXPERIMENTAL DATA

GROUP 1: HEIFERS EXPOSED DURING DIFFERENT STAGES OF PREGNANCY

Twelve heifers of breeding age were included in the first group. Vaginal inoculations were administered at different stages of gestation, ranging from approximately 1½ to 5 months after conception occurred. The data are given in table 1.

[2] FITCH, C. P., chairman. REPORT OF COMMITTEE ON BANG'S DISEASE. Amer. Vet. Med. Assoc. Jour (n. s. 35) 82: 335-344. 1933.

TABLE 1.—*Results with heifers given vaginal injections of Brucella abortus at different stages of pregnancy*

Heifer No.	Date of breeding	Date of exposure	Outcome of pregnancy	Blood agglutinin titer (1 : figure shown) [1]											
				Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
15	Nov. 23	Jan. 14	Aborted July 15.				0	400	1,600	200	800	800	6,400		
17	Nov. 25	do	Aborted Apr. 25.				0	200	800	400	1,600				
18	Dec. 4	do	Aborted June 23.				0	200	3,200	200	3,200	1,600	25,600		
7	June, pasture.	Oct. 5	Aborted Jan. 17.	0	1,600	1,600	1,600								
8	do	do	do	0	1,600	1,600	1,600								
12	do	do	Aborted Jan. 13.	0	1,600	800	400								
66	Jan. 27	Mar. 26, June 20, (eye).	Calved Nov. 11.						0	100	100	50	50	25	50
81	Dec. 6	Mar. 26	Aborted May 27.						0	1,600	3,200	6,400	3,200	1,600	6,400
82	Dec. 5	do	Aborted June 9.						0	1,600	6,400	6,400	6,400	6,400	6,400
24	June 17	Oct. 23	Aborted Dec. 13.	0	800	400	1,600	3,200	3,200	100					
32	June 6	do	Aborted Jan. 21.	0	400	200	1,600	3,200	800	400					
34	May 20	do	Aborted Jan. 31.	0	400	200	6,400	3,200	3,200	3,200					

[1] 0=negative at 1:25. The numerals 25, 50, 100, etc.=complete at 1:25, 1:50, 1:100, etc.

Results.—All the heifers except one (No. 66) developed relatively high agglutination titers (1:200 dilution or above) within approximately 1 month after inoculation. Such agglutination titers persisted as long as the animals were continued in the experimental herd. Each of these 11 heifers aborted. The abortions occurred at various times between the fifth and eighth month of gestation. Heifer No. 66 developed a positive agglutination reaction (1:100 dilution) after approximately 1 month, but within 60 days the titer had fallen to the 1:50 dilution (suspicious reaction). A second inoculation, this time through the conjunctiva, was administered to this animal approximately 3 months after her first exposure. No alteration of the existing suspicious agglutination reaction was observed as a result of this second exposure. This heifer went through a normal gestation and gave birth to a vigorous living calf.

Discussion.—These results strongly suggest that heifers can be infected readily by vaginal inoculation of relatively large doses of virulent *Brucella abortus* organisms. It is important to recognize that under the existing conditions of experimental exposure all possibilities of bacteria entering the animal body through channels other than the vagina were not eliminated. It is conceivable that some organisms may have escaped from the vagina and in some manner have come in contact with other tissues such as the conjunctiva or skin. While such chance transmission of bacteria is possible it seems unlikely that it would have occurred regularly in all of the animals that were muzzled after vaginal inoculations. It seems more reasonable to assume that the infection was the direct result of absorption of the bacteria through the vaginal mucosa.

The results further confirm the generally accepted opinion that the noninfected pregnant heifer usually is highly susceptible to *Brucella*

infections and that the incidence of abortions in infected cattle of this class is high.

GROUP 2: HEIFERS EXPOSED PRIOR TO PREGNANCY

Four heifers of breeding age were included in the second group. Vaginal inoculations were administered at periods ranging from 56 to 75 days prior to breeding. The data are given in table 2.

TABLE 2.—*Agglutinin titers of heifers given vaginal injections of Brucella abortus prior to breeding*

Heifer No.	Date of breeding	Date of exposure	Outcome of pregnancy	Blood agglutinin titer [1] (1 : figure shown)					
				Jan.	Feb.	Mar.	Apr.	May	June
13	Mar. 11	Jan. 14	Calved Dec. 16	0	400	100	200	25	0
14	Mar. 25	...do...	Calved Dec. 8	0	50	50	25	0	0
20	Mar. 8	...do...	Calved Dec. 12	0	100	400	0	0	200
23	Mar. 30	...do...	Calved Jan. 13	0	200	3, 200	400	200	200

[1] See footnote 1, table 1.

Results.—There was no uniformity in the agglutinin development of these animals except that all showed some degree of response, as will be observed in the accompanying data (table 2). The titers were not high consistently and did not persist over a long period. All the heifers had normal gestation periods and gave birth to vigorous, living calves.

Discussion.—The results obtained with this group show a distinct difference in the effects of *Brucella abortus* infection acquired before pregnancy as compared with those observed in the pregnant heifers of group 1.

When infection occurs in heifers at least 60 days before pregnancy the animals usually do not abort. This is consistent with the results frequently reported for heifers inoculated subcutaneously with virulent organisms prior to breeding.

The results further suggest that vaginal inoculations caused sufficient tissue reaction in these heifers to protect them from abortion in the first pregnancy. In other words, these results suggest that it is the status of the animal at the time of infection rather than the channel of exposure that largely determines the results of the infection.

GROUP 3: HEIFERS EXPOSED PRIOR TO BREEDING AND REEXPOSED DURING PREGNANCY

The third group consisted of three heifers approximately 14 months of age. They were therefore beyond the age of puberty but had not reached breeding age at the time of the first exposure. Two of the heifers were exposed through the vagina, the third by way of the conjunctiva, 4, 5, and 7 months, respectively, prior to breeding.

After conception but before the termination of the gestation period, each heifer received two additional exposures to *Brucella abortus*. These subsequent exposures were per conjunctiva except one, which was per vagina. The data, including time intervals and the stage of

pregnancy in each case at the time of these subsequent exposures, are given in table 3.

TABLE 3.—*Heifers exposed to Brucella abortus prior to breeding and reexposed during pregnancy*

Heifer No.	Date of breeding	Date of exposure	Outcome of pregnancy	Blood agglutinin titer [1] (1 : figure shown)																
				Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan
67	Feb. 7	Aug. 18, eye Apr. 26, eye June 20, eye	Calved Nov. 24.	0	1,600	200	100	100	100	50	50	100	100	100	50	50	100	50	50	100	25
77	Dec. 6	Aug. 18, vaginal Mar. 26, eye June 20, eye	Calved Sept. 8	0	1,600	50	0	0	25	0	0	25	25	100	100	100	200	25	25	50	50
80	Jan. 3	Aug. 18, vaginal Mar. 26, vaginal June 20, eye	Calved Oct. 3	0	1,600	50	25	25	25	0	0	50	100	100	50	50	50	25	0	50	--

[1] See footnote 1, table 1.

Results.—All three heifers developed relatively high agglutination titers (1 : 1,600 dilution) within approximately 1 month after exposure. In all three the maximum titers dropped sharply after 1 month. The reinfection exposures to *Brucella abortus* were followed by only relatively slight elevations of the agglutinin titers in some instances and none or decreased titers in others.

All three animals had normal gestations and gave birth to vigorous living calves.

Discussion.—The most interesting feature of this group is the failure of reexposures to appreciably and consistently alter the agglutinin titers or to interfere with normal gestation. It appears that these three heifers acquired a sufficient tissue reaction as a result of exposure prior to breeding to exclude any appreciable effects from subsequent reexposures during the first pregnancy.

GROUP 4: HEIFERS EXPOSED PRIOR TO BREEDING AND REEXPOSED SUBSEQUENT TO BREEDING THAT DID NOT RESULT IN PREGNANCY

The fourth group consisted of four heifers approximately 14 months of age. As in the case of group 3 these heifers were beyond the age of puberty, but were not of breeding age at the time of the initial exposure.

Three of the heifers were exposed through the vagina, one 6 months and the others 7 months before breeding. The fourth was exposed through the conjunctiva, 6 months before breeding. Exposures made subsequent to breeding were administered once in one animal and twice in each of the other three. The intervals and channels of reexposure along with agglutination results are given in table 4.

TABLE 4.—*Heifers exposed to Brucella abortus prior to breeding and reexposed subsequent to breeding that did not result in pregnancy*

Heifer No.	Date of breeding	Date of exposure	Blood agglutinin titer (1 : figure shown) [1]																	
			August	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December	January
69	Feb. 26	Aug. 18, vaginal Apr. 26, Eye June 20, eye.	0	1,600	100	25	50	50	25	0	0	100	50	25	50	100	25	25	25	25
78	Mar. 13	Aug. 18, vaginal Apr. 26, vaginal June 20, eye.	0	1,600	400	3,200	1,600	1,600	100	100	100	50	50	200	100	200	100	100	200	100
89	Mar. 7	Aug. 18, vaginal Apr. 26, vaginal.	0	1,600	50	50	100	100	25	25	25	50	50	50	50	50	50	25	50	...
91	Feb. 24	Aug. 18, eye Apr. 26, Eye June 20, eye.	0	400	400	200	100	100	50	50	25	200	200	50	50	100	25	25	50	...

[1] See footnote 1, table 1.

Results.—The results obtained with this group are essentially the same as those obtained with the preceding group insofar as agglutinin titers are concerned. There were no distinct and consistent alterations in the maximum agglutinin titers following reexposure.

Discussion.—A comparison of the agglutination results obtained with this and the preceding group indicate that the occurrence or absence of pregnancy did not alter the reactivity of heifers that had been infected with *Brucella abortus* prior to breeding. In other words, pregnancy in such heifers did not appreciably change their susceptibility to subsequent reinfection exposures as measured by their agglutinin titers.

GROUP 5: HEIFERS EXPOSED BEFORE THE FIRST PREGNANCY AND REEXPOSED DURING THE SECOND PREGNANCY

Four heifers of breeding age were included in group 5. Vaginal inoculations were administered at periods ranging from 53 to 75 days prior to breeding. Each of the animals was bred and conceived. Agglutination blood tests were conducted at irregular intervals during the first pregnancies of the animals. Four to six months after parturition each of the heifers was bred and pregnancy resulted in all of them. Reinfection exposures were administered during the sixth month of the second pregnancy in three cases and during the fifth month in the other. All received *Brucella* organisms per os on the feed on 3 consecutive days, and two received additional exposures by vaginal inoculation administered 4 days later. The data are given in table 5.

TABLE 5.—*Heifers exposed to Brucella abortus prior to first pregnancy and reexposed during second pregnancy*

Heifer No.	Date of breeding	Date of exposure	Outcome of pregnancy	Blood agglutinin titer (1: figure shown) [1] [2]															
				Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Sept.	Oct.	Nov.	Dec.
13	Mar. 11	Jan. 14, vaginal	Calved Dec. 16	0	400	100	200	25	0	0	0	0	..	0	25	0	0	0	50	50
	June 6	Oct. 23, 24, 25, oral; Oct. 29, vaginal.	Calved Mar. 22																	
20	Mar. 8	Jan. 14, vaginal	Calved Dec. 22	0	100	100	400	0	0	200	50	25	25	100	100	200	200	200	200	100
	May 7	Oct. 23, 24, 25, oral;	Calved Feb. 6																	
14	Mar. 25	Jan. 14, vaginal	Calved Jan. 8	0	50	50	25	0	..	0	0	0	..	0	25	0	0	0	0	0
	May 1	Oct. 23, 24, 25, oral	Calved Feb. 13																	
23	Mar. 30	Jan. 14, vaginal	Calved Jan. 13	0	200	1, 600	200	200	200	100	50	..	200	400	200	200	25	50	25	25
	May 9	Oct. 23, 24, 25, oral.	Calved Jan. 16																	
		Oct. 29, vaginal	Calf weak at birth, survived																	

[1] See footnote 7, table 1.
[2] No data for February to August, inclusive, of the second year.

Results.—Three of the heifers developed positive agglutination reactions (1:100 dilution or above) within 30 days after the initial vaginal inoculations. These titers rather rapidly receded to low positive, suspicious, and even negative zones and fluctuated somewhat durng the first pregnancy. The fourth heifer showed a reaction in only 1:50 dilution for a brief period and then returned to a negative reaction, which persisted throughout the first pregnancy. All four heifers had normal gestation periods and gave birth to vigorous living calves from their first pregnancies.

During the second pregnancy the aggultination titer showed no appreciable consistent alteration following the reinfection exposures. Three of the heifers had normal gestation periods in their second pregnancies and gave birth to vigorous living calves. Heifer No. 23 gave birth to a live calf on the two hundred and fifty-second day of gestation. The calf was weak at birth but survived.

Discussion.—The results obtained with this group should be compared with those of group 3. The essential difference in the two tests is the reexposure of the animals. Initial exposures of each group were made prior to the breeding for first pregnancy. Group 3 animals were reexposed during their first pregnancy, whereas those of group 5 were not reexposed until their second pregnancy.

The general trend of the agglutinin titers did not appear to be materially different in the two groups. The resistance to the act of abortion, presumably induced by the initial exposure of the animals, appeared to be somewhat less complete when reexposure was delayed until the time of second gestation.

TABLE 6.—*Cows exposed to Brucella abortus prior to breeding and reexposed during second pregnancy* [1]

Cow No.	Date of breeding	Date of exposure	Outcome of pregnancy	Blood agglutinin titer [2 3] (1 : figure shown)																
				Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Feb.
2	Feb. 18	Aug. 18, eye Apr. 26, eye June 20, eye	Calved Nov. 27	0	1,600	400	100	100	50	25	50	25	50	50	50	50	100	25	50	100	25
6	Feb. 8	Aug. 18, vaginal Apr. 26, vaginal June 20, eye	Calved Nov. 4	0	1,600	400	100	100	100	25	25	25	100	100	100	25	100	25	25	0	-----
35	Dec. 14	Aug. 18, vaginal Mar. 26, vaginal June 20, eye	Calved Sept. 21	0	1,600	50	25	0	0	0	25	50	25	25	0	25	25	25	-----	-----	-----
3	Feb. 25	Aug. 18, eye Apr. 26, vaginal June 20, eye	Calved Dec. 17	0	3,200	3,200	3,200	1,600	400	200	200	200	400	400	1,600	400	800	200	200	400	200
7	Jan. 2	Aug. 18, vaginal Mar. 26, eye June 20, eye	Calved Oct. 16	0	1,600	200	25	50	50	0	0	200	100	50	50	50	50	25	25	25	-----
64	Mar. 12	Aug. 18, vaginal Apr. 26, eye June 20, eye	Calved Dec. 19	0	1,600	-----	200	200	200	50	50	25	100	200	200	100	100	50	50	50	25

[1] Animals were pasture-bred for first pregnancy; breeding dates unknown.
[2] See footnote 1, table 1.
[3] No data for January of the second year.

GROUP 6: COWS EXPOSED PRIOR TO BREEDING AND REEXPOSED DURING THE SECOND PREGNANCY

Six young cows, each having calved once previously, were included in a sixth group. Virulent *Brucella abortus* organisms were administered per vagina to four of these cows and per conjunctiva to the other two. The animals were not bred until 4 to 7 months after the first experimental exposure. Each received two reinfection exposures during their second pregnancy. Reinfection exposures were administered to the cows during the first half of their gestation periods. Some of the reinfection exposures were per vagina and others per conjunctiva.

Results.—Each of the animals developed a high agglutination reaction within 30 days after the first exposure (table 6). These high titers receded materially within 30 to 90 days. In general the titers dropped to the suspicious or low positive zones (1:25 to 1:100) except for one animal (No. 3), which carried a sufficient titer to be classified as positive every time tests were conducted. The increase in agglutinin titer of the different cows following their first reinfection exposure varied somewhat, but was never so great as that following the initial exposure. The second reexposure did not regularly cause similar titer increases.

All the animals passed through normal gestation periods and gave birth to vigorous, living calves from their second pregnancy.

Discussion.—A comparison of the results obtained with these cows and those obtained with the heifers of group 3, shows no significant difference in agglutinin response attributable to the gestation previous to initial exposure.

The results indicate that these cows developed sufficient tissue reaction from their first exposure prior to their second gestation to protect them against the act of abortion even when subjected to two reinfection exposures during their second pregnancy.

DISCUSSION AND CONCLUSIONS

Many students of Bang's disease have observed that infected cows usually abort but once or twice and thereafter carry their calves full time. In other words, after infection the female bovine usually develops a resistance to the act of abortion after having had the disease for a sufficient length of time to abort one or two calves. It is generally conceded, as a result of clinical experience and infection and vaccination experiments, that some infected cows do not abort. The experiments herein reported confirm these observations. They strengthen the opinion that cows which become infected while they are in a nonpregnant state are not likely to abort their next calves and may never abort. Infection of the heifer or cow sometime prior to pregnancy appears to place her ahead of the animal that is pregnant at the time of infection in that she attains the stage of the disease in which she is less apt to abort without ever having passed through the stage at which animals usually do abort.

It is interesting to note that the results of these experiments are essentially the same as those obtained in vaccination experiments of a past era in which pathogenic cultures were administered subcutaneously to nonpregnant heifers and cows. The results tend to show that it is not the method of administration nor the channel of entrance of *Brucella abortus* organisms, but rather the status of the animal at the time of exposure, that determines what will happen insofar as the act of abortion is concerned.

It should be recognized that these experiments were designed only to study the agglutination response and the incidence of abortion when different classes of cattle were exposed to virulent *Brucella abortus* organisms. The study does not include the question of producing carrier spreaders of Bang's disease. It has long been known that abortion is but one of the symptoms of Bang's disease. If the problems concerned with this disease consisted only in preventing abortions, which is certainly not the case, its solution would be relatively simple since these and other experiments have shown that it is possible to control this symptom to a considerable degree by exposing animals at the proper time.

The results of these experiments strongly suggest that it is relatively easy to infect the female bovine experimentally by placing a suspension of virulent *Brucella abortus* organisms into the vagina. Furthermore, infections through this channel produce effects essentially the same as when infection enters the body through other avenues.

Under the conditions of these experiments, reinfection exposures did not appear to cause definite and consistent alterations in the agglutinin content of the blood of previously exposed animals, nor did they significantly interfere with the animals' capacity to carry their calves to maturity. This should not, however, be interpreted as conclusive evidence as to the role of reinfection in Bang's disease under conditions other than those included in these experiments. Intermittent or continuous reexposure occurring at various intervals before breeding might also affect the outcome of pregnancy.

GROWTH OF MILLET IN QUARTZ SAND AND IN SAND-SOIL MIXTURES [1]

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INTRODUCTION

Data available from previous investigations show the comparative growth made by millet (*Setaria italica* (L.) Beauv.) in quartz sand and in many sand-soil mixtures. With the usual nutrient salts supplied in supposedly optimum amounts, millet in nearly every case yielded more in the sand-soil mixtures than in pure quartz sand. This paper reports the results of experiments that have been conducted to determine how the admixture of a little soil improves quartz sand as a medium for growing millet.

METHODS

Part of the data considered in this paper is taken from previously published experiments dealing with other subjects (*2*, *3*, *4*).[3] The procedure followed in conducting the tests has, therefore, been described in detail and is only summarized here.

Glazed earthenware pots of 1-gallon capacity, holding about 5,000 gm. of sand, were used. The moisture content of the sand or sand-soil mixture, determined by weighing the pots, was maintained at 17 percent (15 percent in some cases) by adding distilled water. Ten millet plants per pot were grown for 23 to 42 days according to the time of year. Usually the heads were about to appear when the plants were cut. The weight varied markedly between plants grown in mid-winter and those grown in early summer.

A uniform mixture of soil or other insoluble material with the quartz sand was obtained by first moistening the sand with 1 percent of water to prevent segregation of materials. The fertilizer salts to be added to the pot were dissolved in the distilled water used to make the sand to moisture content. The fertilizer salts applied were the No. 2 mixture (see table 8), unless otherwise specified.

REVIEW OF LITERATURE

Apparently the effect of soil applications in increasing the yield of sand cultures has not been investigated extensively. Studies have appeared, however, from time to time, dealing with the increased yields produced by adding peat, clay, silica gel, and various nonnutritive solids to quartz sand (*9*).

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[2] Thanks are due E. H. Bailey, of the Division of Soil Survey, for many of the hydrogen-ion determinations considered in this paper, and to Irvin C. Feustel and M. S. Anderson, of the Division of Soil Chemistry and Physics, for furnishing certain materials used in the experiments.

[3] Italic numbers in parentheses refer to Literature Cited, p. 633.

Hellriegel (*5*), the outstanding worker with sand cultures, evidently was not fully satisfied with the yields obtained in cultures of Hohenbockaer glass sand, for at some time in his 30 years' work he commenced adding 5 to 6 percent of washed and acid-treated peat to the sand. This addition is described as being made for the purpose of improving the water-holding capacity of the sand. The peat undoubtedly increased the yields but probably in some way other than through improving the water supply.

In 1915, Koch (*6*) obtained enormous increases, 300 percent and more, in the yields of wheat, rye, oats, and buckwheat, by mixing Zettlitzer kaolin with glass sand in the proportions of 1 to 6. He assumed that the sand-clay mixture could be more readily penetrated by roots than pure sand and attributed the increased growth to this. Ehrenberg et al. (*1*) in the same year reported that clay, peat, diatomaceous earth, and barytes all improved the yield when mixed with quartz sand. Like Hellriegel, they attributed the increased yields to improved water-holding capacity of the mixtures.

A year later Lemmermann et al. (*7*) investigated the beneficial action of the Zettlitzer kaolin in sand cultures. They disposed of the idea that it was concerned with the water supply and showed that it varied with the composition of the nutrient salts. They concluded that the kaolin, which was slightly alkaline, increased the growth of oats and buckwheat by neutralizing the acid reaction of the nutrient salts.

When studying the availability of phosphates, Lemmermann and Wiessmann (*8*) obtained a 50-percent increase in the yield of oats in glass sand by the addition of colloidal silica or calcium permutite, under conditions where the phosphate and other nutrients were supposedly present in excess. The beneficial action of the materials was not explained.

In the related field of water cultures there have been investigations dealing with the effects of adding such adsorptive materials as colloidal silica, humic acid, charcoal, peat, diatomaceous earth, and kaolin. The increased yields that frequently attended the use of these materials have been ascribed to the adsorption of plant toxins and heavy metals or to the maintenance of a more favorable hydrogen-ion concentration.

COMPARATIVE YIELDS IN SAND AND IN SAND-SOIL MIXTURES

Seventy-seven comparisons have been obtained of the yields of millet in quartz sand and in sand-soil mixtures, the fertilizer salts being presumably present in excess. Each sand-soil mixture contained sufficient soil to supply 1 percent of colloid. In 11 cases, involving 7 soils, yields were depressed by the soil additions; but the reasons for the depressed yields are known: Either the soils contained sufficient carbonate of lime to render iron unavailable and bring about a marked chlorosis, or they were markedly acid and fixed so much of the added potassium that yields were reduced by a potassium deficiency. In the remaining 66 cases, involving 46 different samples of soil, yields were increased by the soil applications. The increases ranged from 5 to 162 percent of the quartz-sand yields, the average increase being 56 percent.

A better yield in sand-soil mixtures than in pure sand is evidently not peculiar to millet. Both white mustard (*Brassica alba* (L.) Boiss.) and rice (*Oryza sativa* L.) in single experiments grew considerably better when soil was added to the quartz sand. On the other hand, soil additions that markedly improved the growth of millet did not appreciably affect the growth of Marquis wheat (*Triticum aestivum* L.) as shown in table 1. The values given in this table were taken from two experiments with wheat and two experiments with millet, the data of which have been published previously (*4*).

TABLE 1.—*Wheat and millet compared as to the degree their yields are affected by addition of soil to quartz sand*

Kind of soil mixed with quartz sand	Wheat, yield in soil+sand [1]	Millet, yield in soil+sand [1]
	Percent	*Percent*
Cecil (No. 6977)	107	135
Muskingum (No. B407)	102	137
Colby (No. 6842)	103	[2] 52
Nacogdoches (No. 9475)	108	171

[1] Expressed as percentage of yield in quartz sand.
[2] Millet developed a chlorosis induced by the calcium carbonate present in the Colby soil.

Millet plants grown in pure sand had a slightly different appearance from those grown in sand-soil mixtures and, as will be seen later, it is significant that a difference in the sizes of plants in the two mediums appeared 3 to 4 days after the plants were up. As compared with plants grown in sand-soil mixtures, the plants grown in pure sand seemed less stocky, the leaves were somewhat narrower and shorter but dark green in color, and the whole plant looked more upright and thinner. The most pronounced difference was in the roots. In the pure sand, only a root or two reached the bottom of the 1-gallon pot and the more recently formed roots were thick and crinkled. In the sand-soil mixture, the roots were thinner and much longer, forming a mat at the bottom of the pot. It seems that Koch (*6*) made similar observations on the root growths of plants that he grew in sand and in sand plus Zettlitzer kaolin. While there was a marked difference in the appearance of the roots, weight of roots relative to weight of tops was about the same for the two mediums in a dozen or more cases where the roots were recovered and carefully freed of sand.

In the 66 cases mentioned, where soil additions increased growth by 56 percent on the average, the quantities of soil added to the sand were sufficient to provide 50 gm. of colloid per pot, or about 1 percent of the sand-soil mixture. The effects of larger and smaller proportions of soil in the sand-soil mixture may be seen in tables 2 and 3. The values in table 2 were calculated from previously published data of experiments dealing with other subjects (*3*, *4*). Table 3 gives the results of two experiments with the Wabash subsoil conducted at different seasons of the year.

It is apparent from table 2 that a soil application supplying 50 gm. of colloid per pot was as effective in increasing the yield as applications two to three times as large and that reducing the application to 20 or 25 gm. of colloid diminished the yield appreciably only in the case of the Kirvin soil. In the case of the Wabash subsoil (table 3), a

soil application supplying only 2 gm. of colloid per pot (0.04 percent of the mixture) was plainly beneficial, while an application supplying about 10 to 20 gm. of colloid was about the optimum. Evidently the quantity of soil required to give the best results varies with the kind of soil, just as different soils vary in the increases they produce. Also, the increase produced by one and the same soil often varies considerably in experiments run at different times. The two experiments with the Wabash subsoil reported in table 3 are merely one instance of many that have been observed. Possibly the variations were produced by differences in sunlight at different times of the year.

TABLE 2.—*Yields of millet in sand-soil mixtures containing different amounts of soil relative to yield in pure sand*

Kind of soil mixed with quartz sand	Yield [1] in soil-sand mixture containing indicated amount of soil colloid per pot					
	20 gm.	25 gm.	50 gm.	60 gm.	100 gm.	150 gm.
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Chester (No. 300)	176	----	170	----	----	----
Kirvin A horizon (No. 6679)	111	----	----	144	----	----
Nacogdoches (No. 9475)	----	150	185	----	199	----
Marshall (No. 8736)	----	205	202	----	----	192
Vernon (No. 6718–19)	----	144	156	----	158	----

[1] Expressed as percentage of yield in pure sand.

TABLE 3.—*Comparative yields of millet in quartz sand and in different mixtures of sand and Wabash subsoil (No. 190)*

Experiment and medium in which plants were grown	Air-dry yield of individual pots			Average air-dry yield per pot	
	Grams	*Grams*	*Grams*	*Grams*	*Percent* [1]
Experiment 25:					
Quartz sand only	3.54	3.50	4.42	3.82	100
Quartz sand and soil supplying 40 gm. of colloid	6.61	6.30	6.23	6.38	167
Quartz sand and soil supplying 20 gm. of colloid	6.23	6.16	6.48	6.29	165
Quartz sand and soil supplying 10 gm. of colloid	6.20	5.75	6.53	6.16	161
Experiment 31:					
Quartz sand only	2.90	2.72	2.28	2.63	100
Quartz sand and soil supplying 50 gm. of colloid	6.72	6.50	7.44	6.89	262
Quartz sand and soil supplying 10 gm. of colloid	4.98	4.98	4.84	4.93	187
Quartz sand and soil supplying 5 gm. of colloid	3.82	4.14	3.43	3.80	144
Quartz sand and soil supplying 2 gm. of colloid	4.04	2.78	2.64	3.15	120

[1] Expressed as percentage of yield in quartz sand.

THE QUANTITY AND CONCENTRATION OF SALTS AND THE WATER SUPPLY AS FACTORS IN THE INCREASED YIELD PRODUCED BY SOIL ADMIXTURES

The marked differences in yield in sand and sand plus soil could not have been due to differences in the supply of the principal nutrients. The pure sand and the sand-soil mixtures were given the same fertilization except for the phosphoric acid and the attendant calcium. Sand-soil mixtures made up with soils that render soluble phosphates unavailable were given 1½ to 2½ times as much phosphate as the pure sand cultures to insure an adequate supply. Other sand-soil mixtures containing soils found not to fix phosphates received the same amount of phosphoric acid as the pure sand. Experiments showed that the 0.20 gm. of P_2O_5 applied to the pure sand was more than was needed for maximum growth in that medium.

The quantities of fertilizer salts applied to both cultures (see table 8, fertilizer No. 2) were so large as compared with the available nutrients present in the soil additions that it seemed impossible that the sand-soil mixtures could be at any appreciable advantage over the sand cultures so far as the quantities of available nitrogen, phosphorus, potassium, calcium, magnesium, and sulfate were concerned. Also, the fact that plants in the soil-sand mixtures were larger than those in pure sand a few days after the plants were up showed that small differences in the quantities of these major nutrients could not be responsible for the different growths in the two mediums.

Early investigators attributed the increased yields they obtained with admixtures of 5 to 6 percent of peat or 16 percent of clay to an improved water-holding capacity of the medium. In view of the large quantities of absorptive material added, this does not seem an impossible explanation of their results. But it seems an absurd explanation of the results produced by the much smaller quantities of adsorptive material applied in these experiments. Most of the 46 soils that increased the yield did not sensibly alter the water-holding capacity of the sand when added at a rate to supply 1 percent of colloidal material, and even more certainly the smaller quantities of Wabash subsoil had no such effect.

The pure sand cultures and the soil-sand mixtures were made up to the same water content, about 17 percent, so that if there were a slight difference in available water it was in favor of the colloid-free sand cultures. Moreover, growth in quartz sand was not appreciably affected by marked changes in the water supply. In one experiment, water was added until it ran through a hole provided in the bottom of the pot and the leachings were subsequently used for watering. Under these conditions the sand held temporarily about 28 percent of water instead of the 17 percent normally provided, and the yield of millet was 1.81 ± 0.08 gm. per pot as compared with 1.57 ± 0.13 gm. for the normal conditions.

Related to the water supply is the concentration of nutrient salts applied. This was found not to be unfavorable in the sand cultures. At least, the early growth of millet was the same with one-half the application of salts as with the normal application. The plants, cut before the total quantity of nutrients could affect growth, weighed 0.52 ± 0.02 gm. with the normal concentration and 0.48 ± 0.04 gm. with the half-normal concentration.

TRACE ELEMENTS AS A FACTOR IN THE INCREASED YIELD PRODUCED BY SOIL ADMIXTURES

The possibility that trace elements might be responsible for the beneficial effect of soil additions was tested in a number of experiments. In an experiment previously reported (*2, p. 41*), the yield of millet in quartz sand was 2.10 ± 0.04 gm. per pot with the standard fertilizer and 2.18 ± 0.06 gm. with the standard fertilizer plus copper and boron. Other experiments are reported in table 4. In these experiments, conducted in quartz sand, the standard fertilizer applied was No. 2 (see table 8). The normal applications of trace elements, expressed as grams per pot, were as follows: Copper, 0.00036 gm., as cuprous chloride (Cu_2Cl_2); zinc, 0.0015 gm., as zinc sulfate ($ZnSO_4$); boron, 0.0015 gm., as boric acid (H_3BO_3); and manganese, 0.003 gm., as manganese sulfate ($MnSO_4.2H_2O$).

TABLE 4.—*Yield of millet in pure sand as affected by addition of trace elements*

Experiments and additions to the standard fertilizer	Air-dry yield of individual pots			Average air-dry yield per pot
Experiment 27:	*Grams*	*Grams*	*Grams*	*Grams*
None (check)	2.72	2.33	2.82	2.62
Cu, Zn, Mn, B, normal rate	3.07	2.79	2.95	2.94
Cu, Zn, Mn, B, twice normal rate	2.74	3.10	2.85	2.90
Cu, Zn, Mn, B, four times normal rate	2.72	2.33	2.21	2.42
Cu, Zn, Mn, twice normal rate	2.87	2.43	2.39	2.56
Experiment 29:				
None (check)	2.10	2.54	2.19	2.28
Tap water instead of distilled water	2.40	2.59	2.23	2.41
Tap water+Cu, Zn, Mn, B, normal rate	2.06	2.12	2.20	2.13
Experiment 58:				
None (check)	3.52	2.80	3.68	3.33
0.0008 gm. Fl per pot from NaFl	3.15	3.30	2.70	3.05
0.0040 gm. Fl per pot from NaFl	3.00	3.28	2.78	3.02

It will be seen that trace elements, including fluorine, did not significantly affect the yields obtained in quartz sand. Evidently contamination with containers and impurities in the sand and nutrient salts provided the minute quantities needed.

AN INJURIOUS IMPURITY AS A CAUSE OF THE REDUCED YIELD IN PURE QUARTZ SAND

No evidence having been obtained that the sand cultures were deficient in a mineral nutrient, it seemed possible that an impurity in the sand that would be rendered unavailable by the soil colloids might account for growth being poorer in sand than in sand-soil mixtures. A comparison was therefore made between the ordinary quartz sand and sand purified by acid. About 60 pounds of the sand was digested for 48 hours with 10-percent nitric acid, then washed repeatedly with tap water until neutral in reaction. It was finally washed six times with 5-liter portions of distilled water. The acid evidently dissolved some iron, and the repeated washing with water floated away some very fine quartz powder. Owing to the limited quantity of purified sand available, a preliminary experiment with single pots was conducted before the rest of the sand was used in a second experiment. The results of the two experiments are given in table 5. The soils used in these experiments were applied at a rate to furnish about 1 percent of colloid to the mixture, and the standard fertilizer No. 2 (see table 8) was used. Both the sand and sand-soil mixtures received the same quantity of monocalcium phosphate in these experiments (0.27 gm. of $Ca(H_2PO_4)_2.H_2O$ in experiment 37 and 0.36 gm. in experiment 44), since the Wabash and Marshall soils do not fix phosphates appreciably.

Plants in the purified sand became chlorotic when about half grown, but regained their green color after the addition of another dose of iron, 0.0185 gm. of ferric tartrate per pot. In the second experiment, No. 44, the chlorosis was not allowed to develop so far as in the first experiment. Evidently the poorer growth in purified sand, as compared with that in ordinary sand, was due to a temporary shortage of iron; some iron was present as an impurity in the ordinary sand. The comparative yields in the two kinds of sand, with and without the admixture of soil, gave no evidence of an injurious impurity in the ordinary quartz sand.

TABLE 5.—*Growth of millet in purified quartz sand compared with growth in ordinary quartz sand*

Experiment and medium in which plants were grown	Air-dry yield of individual pots			Average air-dry yield per pot
Experiment 37:	*Grams*	*Grams*	*Grams*	*Grams*
Ordinary quartz sand	1.84	2.27		2.06
Ordinary quartz sand+Wabash subsoil (No. 190)	3.64	3.58		3.61
Purified quartz sand	1.04			1.04
Purified quartz sand+Wabash subsoil (No. 190)	3.67			3.67
Experiment 44:				
Ordinary quartz sand	2.04	1.85	1.85	1.91
Ordinary quartz sand+Marshall soil (No. 8736)	3.53	3.31	3.52	3.45
Purified quartz sand	1.62	1.78		1.70
Purified quartz sand+Marshall soil (No. 8736)	3.44	3.62		3.53

INCREASED YIELDS IN QUARTZ SAND PRODUCED BY MATERIALS OTHER THAN SOIL

Experiments were conducted from time to time to see whether the yield in pure sand could be increased by the admixture of colloidal materials other than soil. The results of these experiments, conducted in 1-gallon pots with fertilizer No. 2 (see table 8), are brought together in table 6.

The material designated as "silicic acid gel electrodialyzed"[4] had been prepared about 1 year before it was used in this study and had a pH value of 3.40 when fresh. The "silicic acid gel commercial" was a dried product of a commercial firm. The "sodium silicate neutralized" was a freshly prepared, unpurified silicic acid gel, made by dissolving sodium silicate in water and adding acid until neutral to litmus. The activated charcoal, as purchased from a commercial firm, was strongly alkaline. It was partly purified by washing repeatedly with distilled water, treating with normal sodium chloride, and then washing until the wash water had a pH value of 7.5 and gave no test with silver nitrate.

The reed peat[5] had a pH value of 4.85. "Reed peat+$CaCO_3$" was the same peat to which sufficient precipitated calcium carbonate was added to give a pH value of 6.81.[6] The iron gel was prepared by approximately neutralizing a ferric chloride solution with sodium hydroxide, washing by decantation 14 times until the gel formed a stable suspension, and then drying at 104° C. The dried material had a pH value of 8.3. All the pots in a given experiment received exactly the same fertilization except the Cecil soil-sand mixture in experiment 29, which received 0.40 gm. of P_2O_5 instead of 0.15 gm., and the iron gel in experiment 58, which received 0.50 gm. of P_2O_5 instead of 0.20 gm.

The dried silica gels, which were quite acid, did not affect the yield significantly, but the freshly precipitated gel (3½ gm. of sodium silicate neutralized) increased the yield about half as much as soil. Ten grams of peat and 10 gm. of activated charcoal gave about equal increases, which were almost as great as that produced by soil. The 10 gm. of ferric oxide gel gave an increase fully equal to that of the soil. All the materials, including the dried silica gels, induced a root growth comparable to that in the soil-sand mixtures. As previously

[4] Prepared by M. S. Anderson.
[5] Furnished by Irvin C. Feustel.
[6] As determined by Irvin C. Feustel.

mentioned, in the soil-sand mixtures there was always a mat of roots at the bottom of the pot, whereas in the pure quartz sand only an occasional root reached the bottom of the pot.

TABLE 6.—*Effect of various colloidal materials on the yield of millet in quartz sand*

Experiment and special materials added to quartz sand	Air-dry yield of individual pots			Average air-dry yield per pot	
	Grams	*Grams*	*Grams*	*Grams*	*Percent* [1]
Experiment 25:					
No addition (check)	3.54	3.50	4.42	3.82	100
2 gm. silicic acid gel electrodialyzed	4.06	4.29	4.79	4.38	115
4 gm. silicic acid gel electrodialyzed	4.02	4.14	4.36	4.17	109
10 gm. silicic acid gel commercial	4.40	3.97	3.64	4.00	105
2 gm. sodium silicate neutralized	3.47	4.23	4.02	3.91	102
Wabash subsoil (No. 190) rate 10 gm. colloid	6.20	5.75	6.53	6.16	161
Experiment 27:					
No addition (check)	2.72	2.33	2.82	2.62	100
3½ gm. sodium silicate neutralized	2.87	3.47	3.64	3.33	127
10 gm. reed peat, pH 4.85	4.12	3.89	3.94	3.98	152
Marshall subsoil (No. 8737) rate 20 gm. colloid	4.05	4.34	3.92	4.10	156
Experiment 29:					
No addition (check)	2.40	2.59	2.23	2.41	100
3½ gm. sodium silicate neutralized	3.42	3.55	3.65	3.54	147
Marshall soil (No. 8736) rate 50 gm. colloid	4.98	4.87	5.03	4.96	206
Cecil soil (No. 6977) rate 50 gm. colloid	3.57	3.80	3.49	3.62	150
Experiment 44:					
No addition (check)	2.04	1.85	1.85	1.91	100
10 gm. activated charcoal pH 7.5	3.06	3.44	3.13	3.21	168
30 gm. activated charcoal pH 7.5	2.72	2.75	2.97	2.81	147
Marshall soil (No. 8736) rate 50 gm. colloid	3.53	3.30	3.52	3.45	181
Experiment 54:					
No addition (check)	1.98	1.93	2.00	1.97	100
10 gm. reed peat	2.30	2.48	----------	2.39	121
10 gm. reed peat + $CaCO_3$	2.43	3.13	----------	2.78	141
10 gm. activated charcoal	2.37	2.55	----------	2.46	125
Experiment 58:					
No addition (check)	3.52	2.80	3.68	3.33	100
9.69 gm. Fe_2O_3 gel dried	6.22	----------	----------	6.22	187

[1] Percentage of yield in quartz sand.

It seems reasonable to assume that these colloidal materials act in the same way as soil in increasing growth. If they do, it is plain that the increased growth produced by soil is due to no ordinary physical change in the medium. Ten grams of activated charcoal or reed peat do not appreciably affect the penetrability, coherence, pore space, or water-holding capacity of 4,800 gm. of quartz sand. Also, if the artificial additions act in the same way as soil in promoting growth, the beneficial effect of soil cannot be due to a growth-stimulating organic compound.

The materials are all adsorptive, the silica and iron gels slightly so, and the activated charcoal and peat to an equal or greater degree than soil, but it is improbable that they change the supply of nutrient ions appreciably in a favorable way. The activated charcoal, after washing, treatment with sodium chloride, and washing, would be expected to exchange only sodium and chloride with the nutrient salts applied to the sand cultures. It is possible, however, that these colloid materials do have a favorable effect on the hydrogen-ion concentration of the medium.

HYDROGEN-ION CONCENTRATION AS A FACTOR IN THE INCREASED YIELD PRODUCED BY SOIL ADMIXTURE

Many of the soils and artificial materials tested did keep the soil-sand mixtures from becoming as acid as the pure sand. Only a part of the increases the materials produced, however, could be attributed to this influence, since some soils that markedly increased growth

gave soil-sand mixtures of the same pH value as the quartz sand cultures. That the hydrogen-ion concentration may be a factor, but not the chief one, in the beneficial effect of soil in sand cultures, is indicated by comparing the pH values of different soils with the increases they produced. Ten soils that had pH values ranging from 7.87 to 6.12 increased growth by an average of 66 percent; seven soils with pH values from 6.05 to 5.52 increased growth by an average of 48 percent; and five soils with pH values of 5.25 to 4.32 increased growth by an average of 49 percent.

The quartz sand used in these experiments had a hydrogen-ion concentration of pH 6.6. The nutrient salts added to the sand and to the sand-soil mixtures are shown as fertilizer No. 2 in table 8. When these salts were dissolved in 800 cc. of water (the quantity added per pot) they gave a solution of pH 3.6. But when the salts were added to the sand the medium had a hydrogen-ion concentration of pH 6.2. After millet had been grown the sand cultures ranged from pH 3.9 to 5.7 in nine different experiments. In earlier work with a similar mixture of salts (magnesium sulfate instead of magnesium chloride and 0.535 gm. of calcium nitrate ($Ca(NO_3)_2.4H_2O$) and 0.338 gm. of potassium chloride (KCl) in place of potassium nitrate) the hydrogen-ion concentration of the sand after growth of millet ranged from pH 3.9 to 5.4 in 10 different experiments. The sand cultures, therefore, became more acid with growth of millet, the hydrogen-ion concentration increasing usually from pH 6.2 to pH 5.0 to 4.5.

The effect of soil additions in modifying the acidity developed by millet in pure sand cultures is shown by the data in table 7. Column 3 shows the hydrogen-ion concentration of pure sand cultures at the end of an experiment; column 4 shows the pH values of sand-soil mixtures in the same experiment; and column 5 shows the pH values of the soils used in making up the sand-soil mixtures.

TABLE 7.—*Hydrogen-ion concentrations developed by millet in sand and in sand-soil cultures, pH values of soils used in sand-soil mixtures, and yield in sand-soil mixtures relative to yield in pure sand*

Experiment No.	Kind of soil mixed with the sand	Hydrogen-ion concentration of—			Yield in sand-soil mixture [1]
		Pure sand culture	Sand-soil culture	Soil used in sand-soil culture	
		pH	*pH*	*pH*	*Percent*
5	Barnes, No. 10305	3.9	6.4	7.2	190
5	Miami, No. 10341	3.9	6.7	6.9	177
5	Davidson, No. 4429	3.9	6.2	6.4	189
6	Carrington soil, No. 10082	5.0	4.8	5.5	179
6	Clarksville soil, No. 195	5.0	5.0	5.4	121
6	Clarksville subsoil, No. 196	5.0	4.8	4.9	167
7	Nacogdoches, No. 5028	4.8	6.3	6.3	164
9	Kirvin soil, No. B299	5.0	5.7	6.1	148
10	Norfolk soil, No. 183	4.5	4.7	4.8	161
10	Norfolk subsoil, No. 184	4.5	4.3	4.3	125
10	Vernon 0–3, No. 6718	4.5	6.1	7.6	153
10	Vernon 3–10, No. 6719	4.5	6.1	7.0	209
10	Vernon 10–27, No. 6720	4.5	6.1	6.4	201
37	Wabash subsoil, No. 190	5.0	5.9	5.8	175
44	Marshall soil, No. 8736	5.8	6.2	6.4	181
60	do	5.5	6.0	6.2	143
61	Marshall soil, No. 8736, heated	5.5	5.1	5.6	198

[1] Expressed as percentage of yield in pure sand.

It will be seen that in sand-soil mixtures the hydrogen-ion concentration is stabilized around that of the soil used in making the mixtures. In most cases the sand-soil mixtures are less acid than the sand culture in the same experiment. But in seven cases the two mediums have nearly the same pH value, and in five of these cases the increased yield produced by the soil admixture is almost as great as when the soil-sand mixture is less acid than the pure sand culture. It seems, then, that regulation of the hydrogen-ion concentration is not the chief factor in the increase produced by mixing soil with quartz sand.

YIELDS IN SAND AND IN SAND-SOIL MIXTURES WITH FERTILIZERS GIVING DIFFERENT HYDROGEN-ION CONCENTRATIONS

Further evidence as to whether the changes in hydrogen-ion concentration could account for the increased growth in soil-sand mixtures was obtained in two experiments. In these experiments comparisons were made of six different fertilizer mixtures that would induce different hydrogen-ion concentrations in the sand cultures. The important variable in the fertilizer mixtures was the ammonium ion, this being chiefly responsible for the acidity developed.

The compositions of the fertilizer mixtures applied are shown in table 8. All mixtures were the same with respect to the quantities of nitrogen, phosphorus, potassium, magnesium, sodium, iron, and manganese present. The quantities of sulfate, chlorine, and calcium varied in some mixtures. The first four mixtures differed from one another chiefly in the relative amounts of the NH_4 and NO_3 radicles. Fertilizers 5 and 6, containing all the nitrogen as NO_3, were nearly alike except that No. 5 contained a neutral mixture of phosphates, while No. 6 contained the alkaline dipotassium phosphate only. No. 2 was the standard mixture used in the experiments reported previously.

Detailed results of the two experiments in which the fertilizer mixtures were used are given in table 9. The same Marshall soil, No. 8736, was used in the two experiments, but in experiment 61 it was heated for 24 hours at 105° C. to kill any root rot organisms that might be present. The unheated soil of experiment 60 had a pH value of 6.15; the heated soil of experiment 61 had a pH value of 5.62. In the treatment "Marshall soil in layers," experiment 61, the soil was mixed with only 10 percent of the sand and this mixture was placed in the pot in four thin layers that were separated by pure sand. The total volume of the layers was about 13 percent of the whole volume of sand in the pot.

In experiment 60 the plants in pure sand receiving the all-nitrate fertilizer No. 6 became strongly chlorotic. In experiment 61, therefore, an extra series labeled "extra Fe" was installed with all-nitrate fertilizer No. 5 to receive extra iron if chlorosis developed. The plants in pure sand receiving this fertilizer did become chlorotic 12 days after planting, and these plants, as well as those in the sand-soil mixture, which were not chlorotic, were then given a dose of 0.0185 gm. of ferric tartrate and 2 days later another dose of 0.0185 gm.

In experiment 60, both the pure sand with fertilizer No. 2 and the sand-soil mixture with fertilizer No. 1 had a pH value of 5.5 after growth and the sand-soil mixture gave a much larger yield (49 percent more). Likewise the sand-soil mixture with fertilizer No. 2 and the pure sand with fertilizer No. 3 had practically the same pH value,

but the sand-soil mixture yielded 37 percent more. In experiment 61, the sand-soil mixture with fertilizer No. 2 had a pH value of 5.1, as compared with pH 5.5 for the pure sand but gave a yield 98 percent greater than the pure sand. These results substantiate the conclusion that change in the hydrogen-ion concentration is only a minor factor in the increases in yield produced by adding soil to sand cultures.

TABLE 8.—*Composition of fertilizer mixtures used in experiments*

Salt used in fertilizer mixtures	Amount of salt applied per pot in fertilizer—					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
	Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
Potassium nitrate, KNO_3		0.93	0.93	0.93	0.499	0.414
Ammonium sulfate $(NH_4)_2SO_4$		.33				
Ammonium nitrate, NH_4NO_3	0.57		.20	.10		
Calcium nitrate, $Ca(NO_3)_2.4H_2O$				.304	1.103	1.16
Potassium sulfate, K_2SO_4	.82					
Magnesium sulfate, $MgSO_4.7H_2O$			.45	.45	.45	.45
Magnesium chloride, $MgCl_2.6H_2O$	.42	.42				
Monocalcium phosphate, $Ca(H_2PO_4)_2.H_2O$	.36	.36	.36	.36		
Dipotassium phosphate, K_2HPO_4					.25	.49
Monopotassium phosphate, KH_2PO_4					.19	
Sodium chloride, NaCl	.05	.05	.05	.05	.05	.05
Ferric tartrate, $Fe_2(C_4H_4O_6)_3.H_2O$	.0185	.0185	.0185	.0185	.0185	.0185
Manganese sulfate, $MnSO_4.2H_2O$	.0015	.0015	.0015	.0015	.0015	.0015

TABLE 9.—*Yields of millet in sand and in a Marshall soil-sand mixture* [1] *with different fertilizers* [2]

Experiment and medium in which plants were grown	Air-dry yield of individual pots				Average yield per pot	Reaction of medium after growth
Experiment 60:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
Fertilizer No. 1+pure sand	2.39	2.18	1.75	2.25	2.14	4.3
Fertilizer No. 1+sand+Marshall soil	3.45	3.60	3.82	4.48	3.84	5.5
Fertilizer No. 2+pure sand	2.69	2.65	2.31	2.66	2.58	5.5
Fertilizer No. 2+sand+Marshall soil	3.35	4.18	2.88	4.29	3.68	6.0
Fertilizer No. 3+pure sand	2.58	2.56	2.80	2.78	2.68	5.8
Fertilizer No. 3+sand+Marshall soil	4.95	4.50	3.35	4.30	4.28	6.8
Fertilizer No. 6+pure sand	2.15	1.98	1.62	1.97	1.53	8.0
Fertilizer No. 6+sand+Marshall soil	4.25	4.05	3.67	4.42	4.10	6.8
Experiment 61: [1]						
Fertilizer No. 2+pure sand	3.27	3.92	3.07	3.51	3.44	5.5
Fertilizer No. 2+sand+Marshall soil	6.69	6.81	6.53	6.95	6.75	5.1
Fertilizer No. 2+sand+Marshall soil in layers	7.35	6.34	7.08	6.95	6.93	5.1
Fertilizer No. 4+pure sand	5.40	4.40	5.10	4.30	4.80	6.8
Fertilizer No. 4+sand+Marshall soil	7.53	6.86	6.35	6.95	6.92	5.9
Fertilizer No. 5+pure sand	2.51	2.62	1.08	2.17	2.10	7.1
Fertilizer No. 5+pure sand+extra Fe	3.07	3.21	2.90	4.65	3.46	7.1
Fertilizer No. 5+sand+Marshall soil	7.55	7.25	7.33	7.68	7.45	6.4
Fertilizer No. 5+sand+Marshall soil+extra Fe	7.17	6.98	6.25	6.18	6.65	6.6

[1] Marshall soil was used at the rate of 50 gm. of colloid per pot. In experiment 61 it was heated for 24 hours at 105° C.

[2] For composition of each fertilizer, see table 8.

The influence of hydrogen-ion concentration on yields in pure sand and in the Marshall sand-soil mixture is shown in table 10, the values for which were calculated from the results of experiments 60 and 61, reported in table 9. Since data obtained in the two experiments may not be exactly comparable, especially in the case of the sand-soil mixture, the Marshall soil having been heated in experiment 61, the values based on experiment 61 are given in parentheses to distinguish them from values based on experiment 60.

TABLE 10.—*Influence of hydrogen-ion concentration on yields of millet in pure sand and in a sand-soil mixture*

Experiment from which data were obtained (No.)	Fertilizer applied	Ammonia in fertilizer [1]	Pure sand		Sand+Marshall soil	
			Yield [2]	Hydrogen-ion concentration after growth	Yield [2]	Hydrogen-ion concentration after growth
	No.	*Mol.*	*Percent*	*pH*	*Percent*	*pH*
60	1	0.00713	83	4.3	104	5.5
60	2	.00500	100	5.5	100	6.0
61	2	.00500	(100)	(5.5)	(100)	(5.1)
60	3	.00250	104	5.8	116	6.8
61	4	.00118	(137)	(6.8)	(100)	(5.9)
61	5	None	(60)	(7.1)	(107)	(6.4)
60	6	None	75	8.0	111	6.8

[1] Expressed as mols NH_4 per pot.
[2] Expressed as percentage of yield obtained with fertilizer No. 2.

Columns 3 and 5 show that the acidity developed in quartz sand is dependent on the quantity of ammonia in the fertilizer. This is also apparent in the sand-soil mixture if the data of experiments 60 and 61 are considered separately.

In the pure sand culture series there is a measurable reduction in yield at pH 4.3 from that obtained at pH 5.5 to 5.8 and a marked increase at pH 6.8. The reduced yields at pH 7.1 and 8.0 are due largely to an iron deficiency produced by the low availability of iron at these hydrogen-ion concentrations. Had iron and phosphate been kept available, yields might have increased at hydrogen-ion concentrations less than pH 6.8. This is shown by the yields of fertilizer No. 5 with "extra Fe" (table 9).

The spread in yields between pH 4.3 and pH 6.8 in the sand cultures is equal to only about one-half the increases produced by soil additions. It therefore seems that regulation of the hydrogen-ion concentration is not the chief factor in the increases produced by mixing soil with quartz sand.

In the sand-soil mixture there is less spread in pH values owing to the buffering effect of the soil colloids. But it also appears as though a given difference in hydrogen-ion concentration is attended by a smaller difference in yields in the sand-soil mixture than in the pure sand. In the pure sand cultures of experiment 61, a 37-percent difference in yield accompanied a change in hydrogen-ion concentration from pH 5.5 to pH 6.8; whereas in the sand-soil mixture, only a 7-percent difference in yield accompanied a change in hydrogen-ion concentration from pH 5.1 to pH 6.4.

SUGGESTED EXPLANATION OF THE INCREASED YIELDS PRODUCED BY MIXING SOIL WITH QUARTZ SAND

If the yield of millet in pure sand falls off more rapidly than the yield in sand-soil mixtures as the hydrogen-ion concentration is increased from near neutrality, as table 10 indicates, the difference in yield between the two mediums should be less near the neutral point than at pH 4.5 to 5.0. Data given in table 9 indicate that this is the case. When the fertilizer applied was No. 4, which gave a pH value of 6.8 in pure sand, the sand-soil mixture yielded 44 percent more than

the pure sand; whereas when the fertilizer applied was No. 2, which gave a pH value of 5.5, the sand-soil mixture yielded 98 percent more than the pure sand.

Furthermore, near the neutral point the smaller increases produced by soil might reasonably be attributed to soil colloids providing available iron in the presence of available phosphates. Millet in pure sand cultures seems to be on the border line of an iron deficiency around pH 6.8, although there does not seem to be a deficiency of iron in plants growing in sand-soil mixtures. This was evident in experiments 60 and 61 (table 9). Here the plants in pure sand receiving fertilizer No. 4 showed a faint chlorosis at times, whereas the plants in sand-soil mixtures did not. Also, there was a marked chlorosis and a greatly reduced yield in the pure sand with fertilizer No. 5, which developed a pH value of 7.1.

Even granting that different yields in the two mediums might have been explained on the basis of iron availability had experiments been conducted with a fertilizer developing a neutral reaction, this still would not explain the 66 cases that have been mentioned where soil admixtures produced an average increase of 56 percent. In these cases, fertilizer No. 2 was used and the hydrogen-ion concentration developed in the pure sand cultures ranged from pH 3.9 to 5.7. At these hydrogen-ion concentrations there was no iron deficiency in the sand cultures, as shown by the dark green of the leaves.

It is believed that in the cases where fertilizer No. 2 was used the roots in the sand-soil mixture were in a less acid environment than in the pure sand cultures and that differences in growth in the two mediums were due to this difference in acidity. This hypothesis is in direct contradiction to conclusious previously drawn concerning the influence of the hydrogen-ion concentration. And it seems contradicted by several instances where the sand-soil mixtures had the same hydrogen-ion concentration as the pure sand cultures. It should be borne in mind, however, that the hydrogen-ion concentrations that have been considered were those of the whole volumes of sand or of sand and soil in the pot, and these data may not be directly pertinent.

Of course it is not the hydrogen-ion concentration of the whole medium that affects the root, but the hydrogen-ion concentration in the water film immediately contiguous to the root. We have no means of measuring the acidity of this film, but presumably it is much higher than that of the whole mass of sand since it is in this film that acidity is developed, following the more rapid absorption of ammonium ions than of nitrate ions. There is some evidence of a higher acidity adjacent to the roots in experiment 61 (table 9), where the soil was applied in four ¼-inch layers separated by 1½ inches of pure sand. The root development was largely concentrated in the thin sand-soil layers. The sand-soil layers and the pure sand layers were sampled separately in some pots, and the hydrogen-ion concentrations were as follows: Sand-soil layers, pH 4.6; sand layers, pH 5.1; and all the material in the pot, sampled as usual, pH 5.02.

Of course, in sand-soil mixtures, as well as in pure sand, the acidity in layers contiguous to the roots should be greater than that of the whole medium; but owing to the buffering effect of soil colloids, the hydrogen-ion concentration of the contiguous sand-soil layers should be less than that of the sand layers.

The hypothesis suggested—that the beneficial effect of mixing soil with sand is due to a reduced acidity in films contiguous to the roots when a fertilizer developing acid is used and is due to the iron supply being maintained when a fertilizer developing alkali is used—is in accord with the experience gained in growing plants in water cultures. A markedly acid reaction depresses growth in water cultures, and when the reaction approaches neutrality growth is likely to be depressed by a deficiency of iron or phosphates. Water cultures and sand cultures differ, however, in that in water cultures, owing to movement of the medium and free diffusion of ions, there is no opportunity for a markedly different layer to develop adjacent to the roots. In sand cultures, it is presumable that a hydrogen-ion concentration developing from an unequal absorption of ions by the roots may, near the roots, temporarily approach the extreme attainable by this process.

SUMMARY

This paper reports the results of an investigation dealing with the growth made by millet in pure quartz sand as compared with the growth made in sand-soil mixtures, the fertilizer salts presumably being in excess in both mediums.

In 66 cases, involving 46 soil samples, millet yielded 56 percent more, on an average, in sand-soil mixtures containing 1 percent of soil colloids than in pure quartz sand. Wheat, however, in 4 comparisons, gave about the same yield in both mediums.

The beneficial effect of soil admixtures on the yield of millet did not increase as the quantity of soil added was increased above that supplying 1 percent of soil colloids. A soil admixture supplying less than 0.2 percent of soil colloids markedly increased the yield.

The greater yield in sand-soil mixtures did not seem to be due to an effect of soil on the water-holding capacity of the medium nor on the concentration of nutrient salts.

The beneficial effect of soil admixtures seemed not to be due to supplying the trace elements copper, manganese, zinc, boron, or fluorine.

The comparatively low yield of pure sand cultures was not due to the presence of an impurity in the sand extractable in 10-percent nitric acid.

Small quantities of peat, activated charcoal, iron gel, and freshly precipitated silica gel, when mixed with quartz sand, produced an increase in yield and a modification in root growth similar to those produced by soil.

Determinations of the hydrogen-ion concentrations of the cultures, after growth and experiments with fertilizers developing different degrees of acidity owing to different proportions of ammonium and nitrate ions, indicate that the beneficial effect of soil does not lie in modifying the hydrogen-ion concentration of the whole medium.

It is suggested, however, that the hydrogen-ion concentration affecting growth is that of the water films immediately contiguous to the roots and that this is probably not shown by a sample of the whole medium. It is probable that when a physiologically acid fertilizer is used an injurious acidity is developed in sand cultures in films contiguous to the roots. In sand-soil mixtures the films are

doubtless less acid, owing to the buffering effect of soil, and the yield is consequently greater. When a physiologically neutral fertilizer is used, sand-soil mixtures should yield more than pure sand, owing to the effect of soil colloids in maintaining the availability of iron.

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